Multimodal Interactions In A Carbonated Beverage System

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Thesis submitted to the University of Nottingham for the degree of Doctor in Philosophy, October, 2007

Acknowledgments

The last 3 years have been quite a journey to say the least, so I'd like to take this opportunity to acknowledge the people who travelled with me.

Firstly, I would like to thank Dr Joanne Hort and Dr Sachin Chandra, my PhD supervisors, for advice and support throughout the course of this project. In particular, to Jo, for offering me an aim, and the chance of a new future at a time when my world had been turned on its head.

To my great mate and mentor, Dr Tracey Hollowood, not only for her ability to instil confidence into a quivering wreck and her ever ready shoulder to lean on, but also for the many, many times we've ended in up in tears through laughing so much.

To be fair, I can't mention laughing until crying without noting the outstanding contribution to this phenomenon of Drs Blissett and Davidson among others (Ian, Av, Jonathon, Melis, Rache, Em, Helen) and, furthermore, for the scientific discussions regarding the categorisation of various biscuit/cake products. To sum up; thanks.

To all of the University of Nottingham's external sensory panel and especially Stuart, Diane, Phil, Sally, Kay, Nina, Sarah, Sue K, Dawn, John, Sue S and Chris, an enormous thank-you for all your hard work in providing me with data to write about! In many ways working in sensory is like having an extended family

and I've so appreciated and enjoyed the friendship, laughter and often bizarre conversations we've shared over the project.

Finally, and most importantly, my greatest thanks go to my family.

To my brothers, Tim, Simon, and Matt, for all the phone calls, and for continuing phoning even when they've had more response from the answer phone!

To Ian, for his love, support and constant faith in me, and his understanding of the trials and tribulations of the write up period!

To my parents, Mum and Chris, for the unending practical and emotional support they have given without complaint, and for the love and care taken in piecing back together a confused, hurt and angry person.

And to my beautiful, caring, funny, sensitive and highly intelligent sons, Jacob and Ben, for providing the inspiration to undertake this challenge; you both make me incredibly proud every single day.

Without you all, quite simply, this just would not have been possible. So this is *OUR* PhD, *OUR* thesis, *OUR* doctorate and I thank-you with all my heart for the opportunities you have given me.

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Abstract

Predicting flavour perception is complicated by interactions occurring both within and across sensory modalities, but understanding these interactions and the resulting multimodal integration is crucial to the formulation of successful products. Despite the commercial appeal of carbonated soft drinks, few studies have examined the effects of *tastant:aroma:carbonation* interactions on sensory perception.

To facilitate these investigations, a citrus flavoured model beverage was created containing ingredients common in commercial beverages; water, aroma volatiles, sugar (glucose or fructose; equi-sweet levels), and acid (citric and lactic acid; equi-sour levels). The complexity of the beverage was gradually increased (influence of carbonation and caffeine) until the model beverage contained elements capable of stimulating gustatory, olfactory and trigeminal systems.

Samples, selected according to D-optimal designs, were evaluated instrumentally (APCI-MS measuring volatile release, rheological measures of viscosity), and sensorially (using a trained panel of assessors). Predictive polynomial models were generated from mean panel data to explain variations in the attributes as a function of the design factors.

The model beverages provided evidence that multi-modal interactions occurred within this model beverage system. Increasing both sugars and acids resulted in an increase in perceived citrus flavour which was not related to any alteration in volatile release measured instrumentally. Intriguingly, glucose and fructose showed different flavour perception enhancement profiles despite being used at perceptually equi-sweet levels. This difference between the monosaccharides was also evident in the predictive models generated for mouthfeel attributes. 'Overall fizziness' was dependant only on carbonation level and unaffected by levels of tastants. However, varying levels of glucose impacted on 'tingling', a relationship not mimicked by fructose.

Addition of carbonation increased perceived sourness, in agreement with previous literature, but results also demonstrated a suppressive effect on perceived sweetness. Interestingly, evaluation of non-caffeinated beverages revealed the perception of a bitter aftertaste, which was primarily driven by CO_2 level, enhanced by citric acid, and suppressed by increasing sugar concentration.

In caffeinated beverages, however, caffeine concentration was the main influence on 'bitterness' and 'bitter aftertaste' attributes. Despite beverage manufacturers including caffeine as 'flavouring' there was little evidence to suggest caffeine concentration modified perception of citrus flavour in this system.

This project provides a comprehensive assessment of the sensory profile of a model carbonated beverage. Combining instrumental and sensorial analysis provided novel evidence of the influence of multi-modal interactions on sensory perception, and highlights the differential effects of two monosaccharides on several key sensory attributes.

1. General introduction

Sales of carbonated beverages dominate the United Kingdom (UK) soft drinks market with a 41.8% share (Zenith International, 2007). These beverages contain water, sweeteners (often high amounts of sugar), acids, aroma volatiles and carbon dioxide, and often include preservatives and colourings. The addition of carbon dioxide adds effervescence and the characteristic fizzy, bubbly mouthfeel associated with such beverages. Regardless of a recent decrease in sales of traditional carbonated drinks, there has been a significant increase in the market demand for 'energy' and stimulant drinks containing elevated caffeine concentrations, with the forecast for a 2007 UK market worth £1billion (Mintel Reports, Energy and Stimulant Drinks-UK-July 2005).

In light of the popularity and widespread consumption of such products, there is a lack of published literature relating to the sensory perception of carbonated beverages, and in particular, the effect of variation of composition (such as altering sugar content) on overall perception. This information is crucial to enable beverage manufacturers to alter formulations whilst retaining key sensory attributes necessary for consumer acceptance. The trend for calorie reduction, replacement of sugars with artificial sweeteners and the growing market for higher caffeine content, require awareness that these modifications may influence more than simply sweet and bitter taste qualities of products. There is a growing understanding of the concept that perception is multimodal in nature and that information from the senses merges to influence sensation (Calvert *et al.* 1998; Calvert 2001; Verhagen *et al.* 2006), a concept discussed in more detail in the following section. Ultimately, understanding the effects of altering any product component on the intrinsic sensory attributes would enable efficient product development to meet consumer's requirements for beverages, including those aimed at specific tasks or situations (dieting, sports, illness).

1.1. Multimodality and perception

Flavour perception is a consequence of detection and processing of gustatory, olfactory and trigeminal stimulation. Several factors, however, can modify the sensations associated with these stimuli. Interactions between gustatory and olfactory systems can occur at a number of junctures, from physical interactions between components within the food matrix to those occurring at a perceptual level. Physical interactions between aroma compounds and other components of the food matrix influencing volatile release have been widely reported (Friel *et al.* 2000; Hollowood *et al.* 2002) and will be discussed in greater detail in Chapter 2. Multimodal perception, however, involves cognitive or psychological integration of the anatomically independent sensory systems. Therefore, the concept of flavour as a multimodal percept involves not only taste and smell, but additional input from the trigeminal system as well as visual and auditory modalities.

Sensory evaluation of both model systems and complex food products, has provided evidence of interactions between taste and aroma stimuli influencing overall flavour perception (Murphy *et al.* 1980; Frank *et al.* 1993; Noble 1996; Stevenson *et al.* 1999). Considerable debate has ensued as to whether these interactions are cognitive in origin, or a consequence of taste-smell confusion or contextual effects, a full review of which is included in Chapter 3. However, studies by Dalton *et al* (2000), Pffeiffer *et al* (2006) and Labbe *et al* (2007), using sub-threshold levels of either tastant or aroma, have produced convincing evidence that cross-modality interactions, between taste and aroma, occur as a result of central processing of sensory information.

Cross-modal interactions have also been reported between the olfactory and trigeminal systems (Schaefer *et al.* 2002; Brand 2006; Verhagen *et al.* 2006; Petit *et al.* 2007). Fibres of the trigeminal system have been shown to innervate the olfactory epithelium and studies suggest that olfactory

responses may be modified by trigeminal activation (Cain *et al.* 1980; Bouvet *et al.* 1987; Kobal *et al.* 1988). Trigeminal stimuli would appear to have an inhibitory effect on olfactory response. Cain and Murphy (1980) reported that the odour of amyl butyrate was diminished in the presence of CO_2, and electrophysiological studies suggest trigeminal stimuli have a suppressive effect on olfactory afferents to the brain (Kobal *et al.* 1988; Inokuchi *et al.* 1993).

Interactions between gustatory and trigeminal systems have also been noted, with both temperature and texture influencing taste (Bartoshuk *et al.* 1982; Cook *et al*, 2003, Lethuaut *et al.* 2003). Furthermore, chemically induced irritation is influenced by the presence of a tastant; sucrose has been shown to suppress the 'burn' of capsaicin (Stevens *et al.* 1986), whilst capsaicin reduces the intensity of sweetness, sourness and bitterness (Lawless *et al.* 1984, Prescott *et al,* 1993). These effects may be due to chemical interactions between the tastants and chemical irritant but, given that the projections from the two systems converge in a number of brain areas, (including the solitary tract nucleus of the medulla (NTS), cortex and thalamus) this raises the possibility of centrally occurring modulation. (Lawless *et al.* 1984; Boucher *et al.* 2003)

Interactions within modalities are also well documented, a phenomenon called 'mixture suppression' was noted as far back as 1961 by Pangborn (1961); when two or more taste stimuli are mixed, the resultant taste intensity perceived was less than the sum of the individual taste intensities. Subsequent studies have identified taste-taste interactions across the five basic tastes resulting in both suppression and enhancement dependant on tastant and concentration (Indow 1969; Breslin 1996; Keast *et al.* 2003).

Neuroimaging studies have provided further support for flavour as a multimodal percept. Rolls and Baylis (1994) reported individual neurons within the primate orbitofrontal cortex (OFC) responsive to both taste and aroma stimuli. Interestingly, these bimodal neurons were most responsive

when the taste and aroma pairing was congruent, i.e. typically experienced together. Subsequent work in human subjects reported activation of the OFC in response to olfactory and taste stimuli. The total activation of the combined stimuli was even greater than the sum of the two presented alone (de Araujo *et al.* 2003; Small *et al.* 2004; Small *et al.* 2005). The OFC also contains unimodal neurons responsive to gustatory, olfactory and somatosensory stimuli, and may provide a site for neural convergence of multiple sensory modalities (Rolls *et al.* 2003; Small *et al.* 2005). In addition, somatosensory inputs relayed through the trigeminal system also converge at the level of the NTS, allowing integration of gustatory and somatosensory information at this early central stage (Boucher *et al.* 2003).

Carbonated beverages provide taste (gustatory), aroma (olfactory), and tactile (trigeminal) stimuli. Although, as discussed, flavour perception would seem to be a consequence of multimodal integration, it is useful to understand how each anatomically separate system may be stimulated by components within foods. The following sections provide a brief overview of the processing of gustatory, olfactory and trigeminal information.

1.2. The gustatory system

On consumption of food, non-volatile substances dissolve in the saliva and are carried to the taste buds and irritant-sensitive regions of the oral cavity. These solutes enter the taste pore (Figure 1-1) where they interact with taste receptors, resulting, through a cascade of intracellular events, in a change in cell membrane permeability. If sufficient, this permeability change results in release of a neurotransmitter and stimulation of action potentials in the axons of sensory nerve cells associated with the taste cells, ultimately relaying information to the gustatory cortex (Sugita 2006).

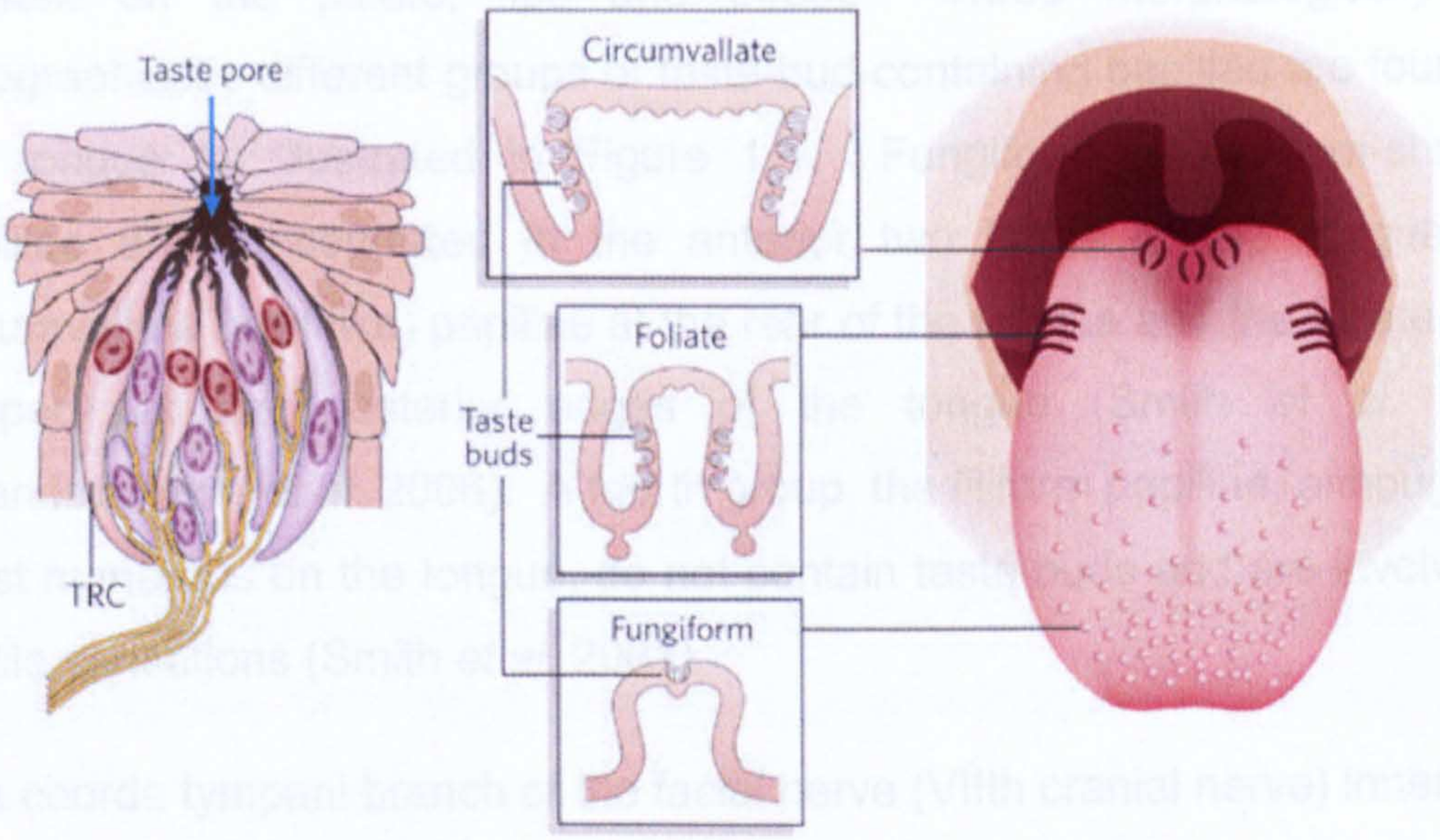


Figure 1-1: Illustration of a) taste bud anatomy and distribution on the tongue (Chandrashekar *et al.* 2006)

Current research agrees that there are five primary tastes: sweet, sour, salty, bitter and umami (savoury). Differential expression of taste receptors provides an argument for topographical taste sensitivity; however, no clear demarcation of the tongue into regions exclusively recognising separate tastes has been established. Each taste bud contains between 50-150 taste cells representative of all five taste sensations, allowing all areas of the tongue to respond to each taste (Hoon *et al.* 1999; Smith *et al.* 2001; Chandrashekar *et al.* 2006). Non-volatile chemicals producing a salty or sour taste appear to act directly, through ion channels, whereas those which produce sweet, bitter and umami tastes act via G-protein coupled receptor sites (GPCRs).

The taste buds themselves consist of three types of cell, specialised epithelial cells which form the supporting capsule, basal cells which differentiate into new taste receptor cells (renewed every 10 days) and taste receptor cells (TRC), found in the interior of the taste bud (Figure 1-1). Each TRC has several microvilli extending into the taste pore which contain the sites (receptors/ion channels) responsible for transduction of taste stimuli.

Taste buds are primarily associated with papillae on the tongue but are also present on the palate, lips and throat. Three morphologically and topographically different groups of taste-bud-containing papillae are found on the tongue as illustrated in Figure 1-1. Fungiform (mushroom-shaped) papillae are concentrated in the anterior two thirds of the tongue, the circumvallate (wall-like) papillae at the rear of the tongue and the foliate (leaf-shaped) at the posterior edges of the tongue (Smith *et al.* 2001; Chandrashekar *et al.* 2006). A fourth group, the filiform papillae, although the most numerous on the tongue, do not contain taste buds and are involved in tactile sensations (Smith *et al.* 2001).

The chorda tympani branch of the facial nerve (VIIth cranial nerve) innervates the anterior and sides of the tongue, whilst the glossopharyngeal nerve (IXth cranial nerve) innervates the posterior portion of the tongue (Boucher *et al.* 2003; Scott 2005). In addition, the Xth cranial nerve provides innervation of the taste buds on the epiglottis and larynx (Scott 2005). These three nerves relay sensory information to the NTS from where it is transferred to the thalamus and onto gustatory cortical areas.

In this project, gustatory stimuli eliciting sweet, sour and bitter tastes were to be used, so each of these are considered further in the following sections.

1.2.1. Sweetness perception

Sweet molecules, such as sugars, artificial sweeteners and some small proteins, bind to a G-protein coupled receptor (GPCR) on the surface of the taste receptor cell. This binding triggers a signalling pathway involving α-gustacin, ultimately resulting in the release of calcium from intracellular stores and cell depolarisation (Lindemann 1996; Margolskee 2002).

The sweet receptor itself has been much studied in recent years and major advances in characterisation and understanding of its make-up and mechanism have been made (Li *et al.* 2002; Damak *et al.* 2003; Spadaccini *et al.* 2003; Jiang *et al.* 2004; Xu *et al.* 2004; Morini *et al.* 2005; Morini *et al.*

2005; Nie *et al.* 2005; Chandrashekar *et al.* 2006; Cui *et al.* 2006; Morini *et al.* 2006). Two members of a family of 3 GPCRs are involved; T1R2 and T1R3, which can assemble into homodimeric and heterodimeric receptor complexes (Nelson *et al.* 2001; Li *et al.* 2002).

Nelson *et al* (2001) performed anatomical mapping studies using immunocytochemistry and *in situ* hybridisation which identified expression patterns for this family of T1R's. Fungiform taste cells contain T1R1 which is always co-expressed with T1R3. Circumvallate, foliate and palate taste cells contain T1R2, again, always co-expressed with T1R3. In addition, there is a non-overlapping population of T1R3-only containing fungiform and palate taste cells.

Transgenic and knock-out (KO) mice studies have identified the T1R1:T1R3 heterodimeric receptor complex as responsible for mediating the umami taste and current research points to the T1R2:T1R3 complex being the predominant sweet taste receptor (Spadaccini *et al.* 2003; Zhao *et al.* 2003; Nie *et al.* 2005; Chandrashekar *et al.* 2006).

1.2.2. Sourness perception

The mechanisms by which acids elicit sour taste are not yet fully understood and the area is complicated by large species diversity (Neta *et al.* 2007). Compounds capable of evoking sour taste are commonly able to dissociate, partially or fully, resulting in production of hydrogen ions (protons). Although proton concentration has been related to sour taste in humans (Gilbertson *et al.* 2000), the correlation between perceived sourness and pH is poor, particularly in cases of incompletely dissociated organic acids (DeSimone *et al.* 2001; Neta *et al.* 2007). In addition, many ion channels, transport proteins and intracellular signalling components are pH sensitive which leads to a wide range of possible transduction mechanisms. It has been suggested that protons act on taste receptor cells (TRCs) in three ways: directly entering the cell; by blocking potassium (K^+) channels on microvilli; and by binding to and

opening other channels on the microvilli that allow other positive ions into the cell (Rawson 2004). More recent evidence presented by Huang *et al* (2006) and Ishimaru *et al* (2006), has identified PKD2L1 (a polycystic-kidney-disease-like ion channel) as a candidate sour taste sensor. Both studies reported expression of PKD2L1 in all papillae type but noted a lack of co-expression with T1R's and T2R's, taste receptors responsible for sweet, umami or bitter tastes. Huang and colleagues also generated mice deficient in PKD2L1 and demonstrated complete elimination of response to acids in these animals, whilst responses to bitter, sweet, salty and umami tastants were unaffected.

1.2.3. Bitterness perception

Current evidence suggests the receptors mediating bitter taste, in common with those mediating sweet taste, are a family of GPCRs. These have been termed T2R's and gene studies suggest humans express ~25 different potential bitter receptors (Adler *et al.* 2000; Andres-Barquin *et al.* 2004; Behrens *et al.* 2006; Chandrashekar *et al.* 2006).

Anatomical mapping studies have determined expression of multiple T2Rs in approximately 20% of taste cells in both foliate and circumvallate but rarely fungiform papillae (Hoon *et al.* 1999). As is the case for the sweet receptor, T2Rs are partially coexpressed with α-gustacin, and mice lacking this show reduced sensitivity to bitter compounds (Sugita 2006). This suggests a common signalling pathway for bitter and sweet taste transduction (Zhang *et al.* 2003).

Genetic variation in human bitter taste has been observed and linked to polymorphisms on several T2R genes; the most widely studied being those on T2R38 which confers sensitivity to the chemical compounds phenylthiocarbamide (PTC) and 6-*n*-propylthiouracil (PROP) (Kim *et al.* 2003). This variation in human bitter sensitivity has been linked to sensitivity for other taste qualities (Gent *et al.* 1983; Yackinous *et al.* 2001; Prescott *et*

al. 2004), dietary choices and consequential health implications (Duffy *et al.* 2000; Duffy *et al.* 2004; Tepper *et al.* 2004).

1.3. ***The olfactory system***

Olfaction, the sense of smell, occurs in response to aroma volatiles entering the extreme superior region of the nasal cavity and interacting with the olfactory neurons of the olfactory bulb. This can happen in two ways; orthonasally, where aroma volatiles enter via the nostrils, or external nares (on sniffing), or retronasally, where aroma volatiles enter via the internal nares from the oral cavity (during swallowing).

Once in the nasal cavity, the aroma volatiles dissolve in a thin film of epithelial mucus and interact with the olfactory vesicles either directly or via binding to olfactory binding proteins (OBPs) (Pevsner *et al.* 1988; Snyder *et al.* 1989). The olfactory vesicles are the end of the dendritic projections of olfactory neurons. These bipolar neurons project through small holes in the bony cribriform plate to the olfactory bulbs (Figure 1-2), with the olfactory tracts extending between the bulbs and olfactory cortex (Bear 2007). Olfactory information is then transferred to higher cortical areas and the limbic system.

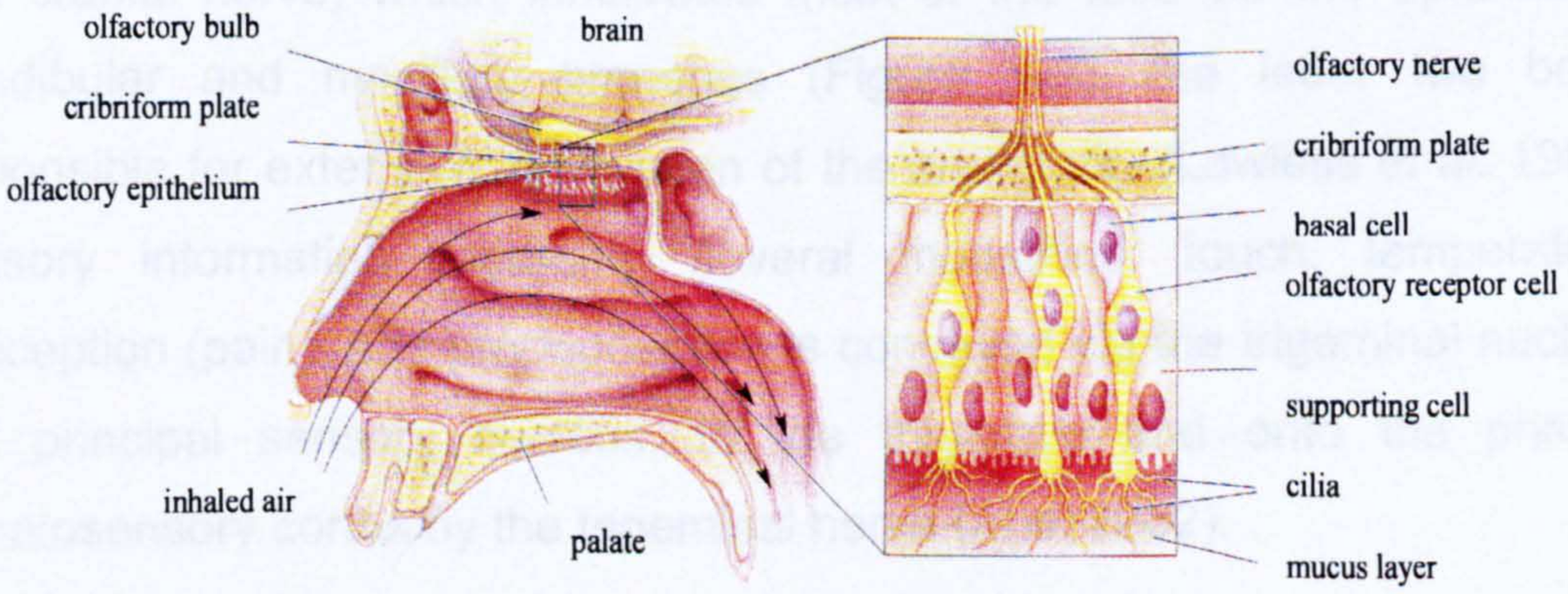


Figure 1-2: Location and structure of the olfactory system (source: Bear *et al* 2007)

The olfactory neurons have a high turnover rate, regenerating approximately every two months, and each is able to respond to more than one type of aroma volatile. When an aroma volatile binds to the olfactory receptor, an IP_3 2^{nd} messenger signalling cascade is triggered, resulting in depolarisation of the cell and generation of an action potential (Buck 2004) Different volatiles are recognised by different combinations of receptors which results in generation of unique 'aroma codes'. In humans, a GPCR family of about 350 olfactory receptors appear to be employed in a combinatorial manner, enabling detection of over 100,000 aroma volatiles (Buck 2004).

In terms of consumption of food, both orthonasal and retronasal stimulation of the olfactory system may occur and the actual physical release of aroma volatiles from the food matrix is governed by a number of factors. The most significant factors are: concentration of volatiles; their spatial location and availability (free, entrapped, adsorbed etc); presence of other components (lipids, carbohydrates, proteins, water); amount of saliva in the mouth; temperature and pH (Taylor 2002).

1.4. The trigeminal system

Processing of somatosensory information occurs via the trigeminal nerve (Vth cranial nerve) which innervates most of the face via the ophthalmic, mandibular and maxillary branches (Figure 1-3), the latter two being responsible for extensive innervation of the oral cavity (Lawless *et al.* 1984). Sensory information regarding several modalities: touch, temperature, nociception (pain), and proprioception is conveyed via the trigeminal nucleus and principal sensory nucleus, to the thalamus and onto the primary somatosensory cortex by the trigeminal nerve (Abdi 2002).

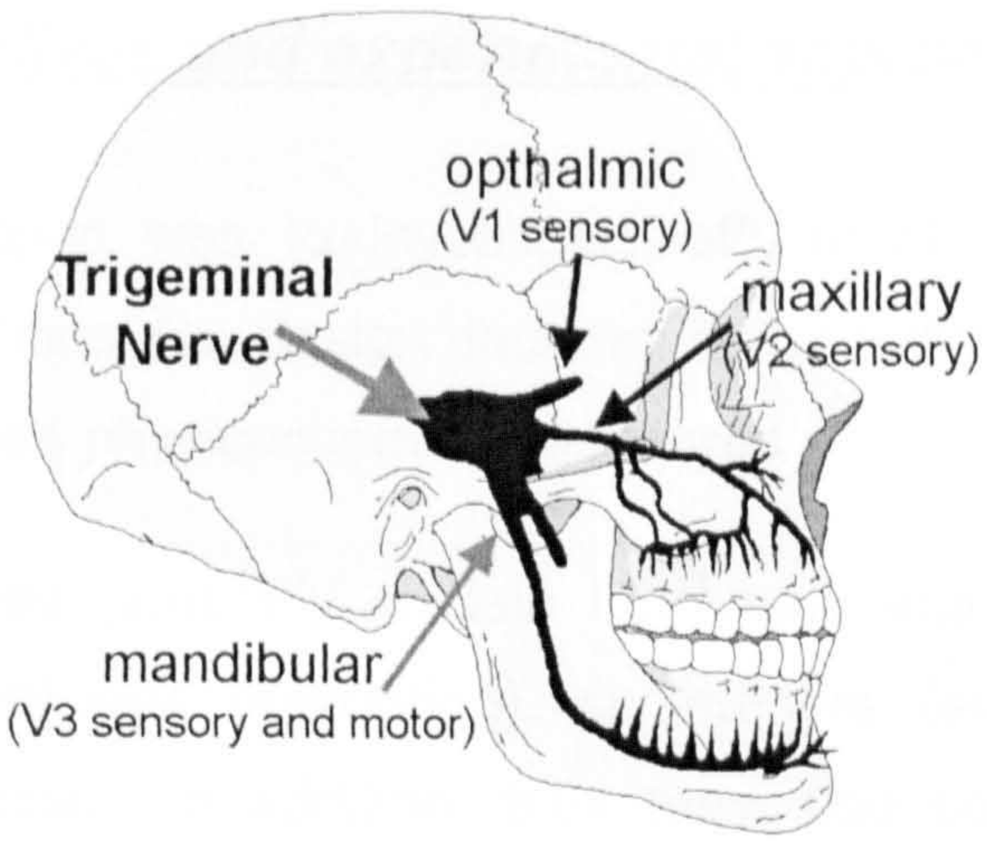


Figure 1-3: Innervation of the face by the trigeminal nerve and its branches (source: Oregon Health and Science University).

It is not only the texture, temperature and consistency of food that is conveyed by the trigeminal system, as it is also stimulated by a number of chemical substances. These substances include alcohol, menthol, carbon dioxide, capsaicin and high concentrations of aromas (Liu *et al.* 1998; Dessirier *et al.* 2001; Bryant *et al.* 2002; Carstens *et al.* 2002; Brand 2006 Prescott and Stevenson, 1995). The sensations elicited are often described as burning, cooling, tickly, stinging, warming or painful, and one of the functions of the trigeminal system seems to be protection from the effect of harmful substances. Many of these attributes, however, are sought after in food products and, at low levels, considered pleasant by consumers; capsaicin, the active ingredient in chilli peppers, being a good example of this. Capsaicin stimulates the trigeminal system via activation of ion channels on heat and pain sensitive nerve fibres (Carstens *et al*, 2002).

Carbon dioxide is regarded as an odourless trigeminal stimulus, with limited taste qualities in solution, and is widely used in the food industry to add effervescence to beverages. CO_2 stimulates the trigeminal system not only via activation of mechanoreceptors within the oral mucosa but, in addition, CO_2 elicits a chemogenic response, following its conversion to carbonic acid (Simons *et al*, 1999, Dessirier *et al*, 2000). The mode of action of CO_2 on the trigeminal system is discussed in further depth in Chapter 4.

1.5. Aims, objectives and experimental approach

The aim of this project was to investigate effects of varying the main ingredient concentrations (or design factors) in beverages on perceptual responses and selected physicochemical measures.

Commercial beverages generally contain four main ingredients; water, a cocktail of aroma volatiles (flavouring), sweeteners (sugars or artificial sweeteners), and acids. In addition, they may also contain CO_2, other tastants (caffeine), preservatives, and colouring. Controlling this number of factors is prohibitive when designing experiments to understand the influence of individual factors together with interactive effects. Consequently, a model system was created using ingredients most commonly included in commercial products; water, aroma volatiles, sugar, and acid. The use of a model system allows design factors to be varied as necessary, but does have limitations (for example, palatability) compared to more complex commercial beverages.

1.5.1. Model beverage system

The model beverages developed for the study were initially very simple: a combination of one sugar and one acid, together with a blend of two aroma volatiles. After sensory and instrumental evaluation of these systems, the level of complexity was increased layer by layer, building on the conclusions from preceding investigations. Ultimately, the model beverages contained elements capable of stimulating gustatory, olfactory and trigeminal systems. Controlling compositional variation allowed the investigation of effects on perceptual attributes and expanded current knowledge regarding influences of multimodal interactions.

1.5.2. Experimental design

To facilitate these studies, a Design of Experiments (DOE) approach was adopted. This provided a systematic approach to investigating the beverage

system and allowed the influence of varying the design factors (independent variables), and their interactions, on sensory properties (dependant variables) to be assessed. Response surface methodology (RSM) allowed quantification of the relationships between measured sensory responses and design factors. The response surface characterises the main effects and interactions between variable design factors, and can be represented by polynomial models and visualised by contour and interaction plots. Classical designs, such as factorial and central composite designs, with several variable factors often result in impractically large numbers of samples for sensory testing. Therefore, to limit the number of samples for sensory assessment, computer generated D-optimal designs were employed (Design Expert). D-optimal designs select a smaller subset of samples whilst minimising the variance of the model coefficients. These designs are able to provide reliable modelling of sensory responses using this subset of the total number of potential samples, but still allow inclusion of experimental replicates (deAguiar *et al.* 1995).

The samples selected by the D-optimal designs were evaluated by a trained panel of expert sensory assessors. Initially the panel used the technique of magnitude estimation (Chapter 3) and, subsequently, moved on to sensory profiling to evaluate the more complex carbonated and caffeinated beverages (Chapters 4 and 5). Expert assessors were essential for these experiments as they had a wealth of previous experience of deconstructing flavour into separate components of taste and aroma. Following assessment of panel performance, raw data were analysed using ANOVA and multiple comparison tests, and mean panel data using principal component analysis, to assess the influence on perceptual responses of variation in each design factor. Finally, multiple linear regression was used to generate predictive models, and provide interaction and contour plots to visualise data and aid interpretation.

A dual experimental approach was taken, combining sensory evaluation with instrumental analysis. The effect of varying the design factors on volatile release and viscosity of samples was examined using atmospheric pressure chemical ionisation mass spectrometry (APcI-MS) and rheometry respectively (Chapter 2). Evaluation of these physicochemical parameters allowed assessment of chemical or physical changes in the beverage matrix which could potentially influence sensory perception. These instrumental measurements provided key information for interpreting sensory data and identified whether modification in sensory perception, as a response to variation in beverage composition, resulted from physicochemical interactions rather than as a consequence of cross modal integration within the brain.

1.5.3. Layout of the thesis

In order to provide a report which followed the transition through the experimental approach taken, a more detailed introduction is integrated within each of the following four experimental chapters. In addition, each chapter also contains an individual discussion section allowing consideration of findings pertinent to the subsequent studies.

Studies reported in Chapter 2 examine the effect of beverage composition on the most relevant instrumental measures, to determine the extent of physicochemical interactions within the beverage matrix and identify those with the capability of directly influencing sensory properties.

Chapters 3, 4 and 5 focus on sensory evaluation of the model beverages, through the stages of complexity, and the influence of variation in constituent concentration on sensory perception. Chapter 3 examines taste and aroma interactions including the effects of different sugars and acids. Chapter 4 details the influence of incorporating carbonation into the model beverages, and Chapter 5, the influence of caffeine on sensory attributes.

Finally, Chapter 6 provides an overview of the projects general conclusions, the major findings, implications and further work.

2. Physicochemical interactions

2.1. Introduction

The release of flavour molecules from a food product during consumption provides one of its key sensory attributes. These flavour molecules are volatile components contained within the food matrix which are released into the gaseous phase and interact with the nasal odour receptors.

As detailed in the previous chapter, this can occur through two routes: orthonasally and retronasally. In both conditions, a number of factors can effect the distribution of the volatile molecules between the matrix and gaseous phases. Volatile release from the carrier medium and delivery to the nasal cavities is dependant on the chemical nature and concentration as well as spatial location and availability (free, entrapped) of the volatile(s) in question.

Bulk phase physical parameters, such as activity coefficients, partition coefficients, and mass transfer between aqueous and gaseous phases vary between aroma volatiles and are susceptible to influences from matrix constituents (Voilley *et al.* 1977; Lethanh *et al.* 1992).

The activity coefficient is a measure of the degree of compatibility of the volatile with the aqueous phase, i.e. the affinity of the volatile for the carrier medium compared to the gaseous phase above it. This measure describes the intermolecular interactions between volatiles, solutes and solvents and as such may be modified by alterations in composition of the aqueous phase (Lethanh *et al.* 1992; Taylor 2002).

The mass transfer coefficient is a measure of the rate of release of an aroma volatile from the matrix. Mass transfer between the aqueous and gas phase is associated with a diffusion coefficient which in turn is determined by the viscosity and water activity of the aqueous phase (Nahon *et al.* 2000).

The distribution of aroma molecules between matrix and gaseous phase in a closed system at equilibrium at a constant temperature is described by the partition coefficient. The partition coefficient at equilibrium is defined in Equation 1.

$$K^i = c_g^i / c_p^i$$

Equation 1: Partition coefficient at equilibrium

Where: K^i is the partition coefficient, c_g^i and c_p^i are the gas and product phase concentration respectively, of the aroma compound *i*, at equilibrium

In an aqueous system such as a water-based beverage, aroma volatiles are present at extremely low concentrations and can be considered 'infinitely dilute' (Taylor 2002). Therefore, the partition between the two phases obeys Henry's Law: "the mass of vapour dissolved in a certain volume of solvent is directly proportional to the partial pressure of the vapour that is in equilibrium with the solution" (Taylor, 1998). Simply, in an equilibrium state, the concentration of a volatile compound in the static headspace (the gaseous phase) is directly proportional to the concentration in the aqueous phase.

However, the volatility of aroma compounds may be influenced by altering the physical or chemical properties of the aqueous phase. Presence of components within the aqueous phase can result in direct interaction between volatiles and components which can be either repulsive or attractive (Lethanh *et al.* 1992; Goubet *et al.* 1998). Equally, constituents within the aqueous phase may modify the solvent properties for example by changing the ionic environment, and so alter volatility indirectly (de Roos 1999; Deibler *et al.* 1999).

2.1.1. Effect of solutes on volatile release

The presence of solutes, such as sugars or salts, within a solution has been shown to affect the relative partitioning of the volatiles and their molar concentration (via hydration changing the amount of 'free water') as well as

affecting the activity coefficient (Lethanh *et al.* 1992; Friel *et al.* 2000; Nahon *et al.* 2000; Hansson *et al.* 2001; Taylor 2002). Studies by Voilley (1977) and others (Massaldi *et al.* 1973; Massaldi *et al.* 1974; Ebeler *et al.* 1988; Nahon *et al.* 1998; Nahon *et al.* 2000; Da Porto *et al.* 2006) suggest that interactions between solute and volatile are dependant on the nature of the volatile. These groups examined effects of addition of sucrose and other sweeteners on headspace partitioning of a range of volatiles. Results indicated that headspace volatile concentrations may decrease, increase or remain the same depending on the nature of the volatile examined and the solute type. This phenomenon is often referred to as 'salting out' in the case of increases in headspace volatile concentration and 'salting in' when the headspace concentration is reduced.

As detailed, a number of studies have examined the impact of sugars on volatile release into the headspace phase. Complimentary results have been reported by studies examining the influence of salts (Ebeler *et al.* 1988) and acids (Voilley *et al.* 1977; Reynolds *et al.* 1982; Marsh *et al.* 2006) on the partitioning of volatiles in liquid based systems.

2.1.2. Effect of viscosity on volatile release

As previously described (2.1), the rate of mass transfer of volatiles between the gas and aqueous phase is governed by the rate of diffusion of the volatiles within the phases. This diffusion is related to the viscosity of the solution, and as viscosity increases the rate of diffusion of a volatile molecule decreases (Roberts *et al.* 1996). Moreover, volatility of aroma molecules may also be affected by the specific thickener used as a result of direct binding interactions occurring between the two. Roberts *et al*, (1996) investigated flavour release in solutions thickened to equi-viscosity with three different thickeners (sucrose, guar gum and carboxymethylcellulose). Their findings indicated differing volatile release profiles between the solutions and they concluded that both viscosity and specific binding of aroma compounds within the matrix contributed to these effects.

The viscosity of a solution does not solely impact on flavour perception through physical interactions with the aroma volatiles but additionally brings the sensation of altered mouthfeel or texture. Of particular relevance to this thesis, is the finding that viscosity has previously been shown to impact on not only perception of flavour, but also non volatile attributes such as sweetness and sourness (Malkki *et al.* 1993; Walker *et al.* 2000; Lethuaut *et al.* 2003).

The studies described within this thesis focus on identifying the presence of multimodal interactions within a model beverage system and the investigations in subsequent chapters concentrate on sensory evaluation to describe the perceptual result of these interactions. However, the ability of the constituents within the model systems to affect chemical changes, influencing aroma release, or interactions resulting in physical alteration of the beverage matrix should not be underestimated.

Previous literature studies have shown physical and chemical interactions within a food or beverage matrix can occur which impact on sensory perception (Lethanh *et al.* 1992; Landy *et al.* 1995; Goubet *et al.* 1998; Duran *et al.* 1999; Lubbers *et al.* 2001; Hollowood *et al.* 2002; Hollowood *et al.* 2002; Lethuaut *et al.* 2005; Da Porto *et al.* 2006; King *et al.* 2006).

These studies often report findings in systems containing a much greater concentration of solutes than encompassed in the model beverage system used in the present study, often up to 65% sucrose (Friel *et al.* 2000). Nonetheless, in undertaking investigations into the influence of multimodal interactions on perception, it is important to identify if and how, interactions occur at a physico-chemical level within the model system.

To this end, the most relevant physico-chemical interactions in the model beverage system were quantified by measurement of rheological parameters and measurement of headspace volatile concentration. Headspace analysis is commonly used in the food industry to identify relative volatile contents and

in the detection of alterations in such resulting from modification of the food matrix.

2.1.3. Instrumental measurement of headspace volatile release

The relationship between volatile concentration in the gaseous and aqueous phases (Equation 1), can be exploited by measuring changes in the headspace volatile concentration (in a static system), and use this data to signify changes in volatile concentration or availability in the aqueous phase. In this way, changes in the partitioning of aroma volatiles as a result of variation in solutes within the aqueous phase can be determined. Measurement of the headspace volatile concentration traditionally was achieved by the collection of a sample of the headspace at equilibrium, commonly by use of Tenax traps or coated fibres (Solid Phase Micro Extraction, SPME) (Da Porto *et al.* 2006). The volatile molecules are then separated by gas chromatography and the individual molecules quantified by mass spectrometry.

An alternative to this method is atmospheric pressure chemical ionisation mass spectrometry (APcI-MS). APcI-MS was developed by Taylor and Linforth (2000) to measure volatile release in real time. This technique allows headspace sampling directly into the mass spectrometer via a novel interface. The sample is drawn into a heated, fused silica capillary tube by means of a venturi effect created by a high nitrogen gas flow. The volatile compounds are then ionised by a corona discharge pin (positive ion mode, 4kV) and the resultant ions protonated, by transfer of charge from protonated water, before entering the high vacuum region of the MS analyser. This 'soft' ionisation method reduces the amount of fragmentation occurring, hence the identification of volatiles from the resulting mass spectra is uncomplicated (m/z; effectively the molecular weight of the volatile molecule plus 1 to account for the proton transfer).

APcI-MS samples only a small volume of headspace, thereby reducing the disturbance to the equilibrium state and removing the need for adsorption of volatiles onto traps or fibres prior to analysing. The resultant MS chromatogram rapidly stabilises, therefore each headspace analysis only requires a short sampling time (commonly <30secs). These advantages allow for very fast sample comparison compared to the GC-MS techniques.

The objective of the present study was to examine the effect of addition of tastants on the partitioning of two volatile aroma compounds; citral and limonene. These terpenes are perceptually characterised as lemon/lime and lemon/orange odours and together provide a citrus-style flavouring. The two volatiles form the flavour base of a water-based model beverage system designed to investigate multimodal interactions and their impact on flavour release and perception. The effect of varying levels of four tastants; citric acid, lactic acid, glucose, or fructose, on two concentrations of the aroma compounds, was examined by measurement of static headspace volatile concentration at equilibrium (using APcI-MS). Unfortunately, levels of aroma volatiles in exhaled breath following consumption of model beverage samples were below the level of sensitivity for APCI-MS measurements to be made 'in-nose', so direct measures of volatile concentration at the level of the odour receptors was not possible.

This study was expanded to examine the influence of carbonation on partitioning of volatile components within the model beverage, both alone and in the presence of tastant. Once more, headspace volatile concentration at equilibrium was measured. However, in order to simulate volatile release profiles on first opening a carbonated beverage, an additional measure of headspace volatile concentration was made immediately after opening samples.

Finally, the effect of the four tastants on the viscosity of the aqueous system was assessed using rheological measurements.

2.2. ***Materials and Methods***

Citral (3,7-dimethyl-2,6-octadienal) and limonene (1-methyl-4-prop-1-en-2-yl-cyclohexene) (Aldrich, Dorset, UK), combined together in water (Brecon Carreg. UK), produced a citrus style flavouring used as the basis of the model beverage.

Four tastants; citric acid (99% Lancaster Synthesis, Lancaster, UK), lactic acid (85+% solution in water, Sigma, USA), glucose (99+% Fisher Scientific, Loughborough, UK), or D(-)-fructose (98% Acros Organics, USA), were investigated at concentrations comparable to commercial beverages.

Carbonation was achieved using laboratory carbonating apparatus (food grade CO_2, BOC, UK). Samples were carbonated to a level of 3.6volumes CO_2/L, which is comparable to commercial carbonated beverages.

2.2.1. Experiment 1: Effect of solutes on volatile release

2.2.1.1. Sample preparation

Two levels of aroma volatiles were identified which resulted in solutions with a perceivable difference in citrus flavour (aroma level 1: 2.5ppm of each volatile, aroma level 2: 10ppm of each volatile). The effect of tastants on volatile release was examined for each level.

Samples were prepared containing varying amounts of citric acid (0, 0.5, 1.0, 1.5g/L), lactic acid (0.9, 1.8, 2.6ml/L), glucose (50, 100, 150g/L) or fructose (20, 40, 60g/L) dissolved in mineral water. The aroma volatiles were dissolved in propylene glycol (Fischer Scientific, UK), vigorously shaken at 700 oscillations/min (Stuart Scientific Flask Shaker, UK) for 20mins and added to samples to obtain a final concentration of either 2.5ppm each citral and limonene (aroma level 1) or 10ppm each citral and limonene (aroma level 2). All samples were subsequently mixed on a roller bed for a minimum of 1hr to ensure all components were fully dissolved and dispersed.

2.2.1.2. *Headspace analysis of volatile release*

Assessment of headspace volatile concentration by APcI-MS was used to investigate the effect of alterations of non-volatile components in this system on physical release of flavour volatiles.

Replicate samples (5 for each condition) were aliquoted into sealed glass bottles (40ml sample in 100ml bottle) and left for 2hr at ambient temperature to allow equilibrium between the aqueous phase and the gaseous headspace to be reached. The static headspace (5 replicates) was sampled into a Platform II Mass Spectrometer fitted with an APcI source developed in-house (by Prof A. Taylor and Dr R. Linforth) and commercially available as MS-Nose^{TM} (Micromass, Manchester, UK) interface. Sampling was executed for approximately 30s at a flow rate of 10-12 ml min^{-1} and was performed in a randomised order to account for any fluctuations in instrument sensitivity over the time course of the experiment.

Limonene (M.wt. 136) and citral (M.wt. 152) were monitored in selected ion mode (m/z 137 and 153 respectively, dwell time 0.5s). Comparison of peak height data (% peak intensity) obtained from chromatograms analysed using MassLynx software (Micromass Ltd, UK) allowed mean relative amounts of each compound to be determined and compared across the ranges of solute variation (Friel *et al.* 2000).

2.2.2. Experiment 2: Effect of carbonation on volatile release

2.2.2.1. *Sample preparation*

Samples were prepared containing citric acid (0g/L, 1.5g/lL, glucose (150g/L) or fructose (60g/L) dissolved in mineral water. To each sample, aroma volatiles were added to give a final concentration of 2.5ppm each of citral and limonene. All samples were subsequently mixed on a roller bed for a minimum of 1hr to ensure all components were fully dissolved and dispersed.

2.2.2.1.1. Carbonation of samples

Following roller bed mixing, duplicate samples were stored at 4-6°C prior to carbonation. A method of carbonating samples was constructed using a cylinder of food grade CO_2 (BOC, U.K.) connected, via a regulator, by plastic tubing to a bottle lid (see Figure 2-1). The flow of CO_2 could be isolated by means of a two-way switch and a pressure gauge was fitted to allow the pressure in the sample bottle to be monitored.

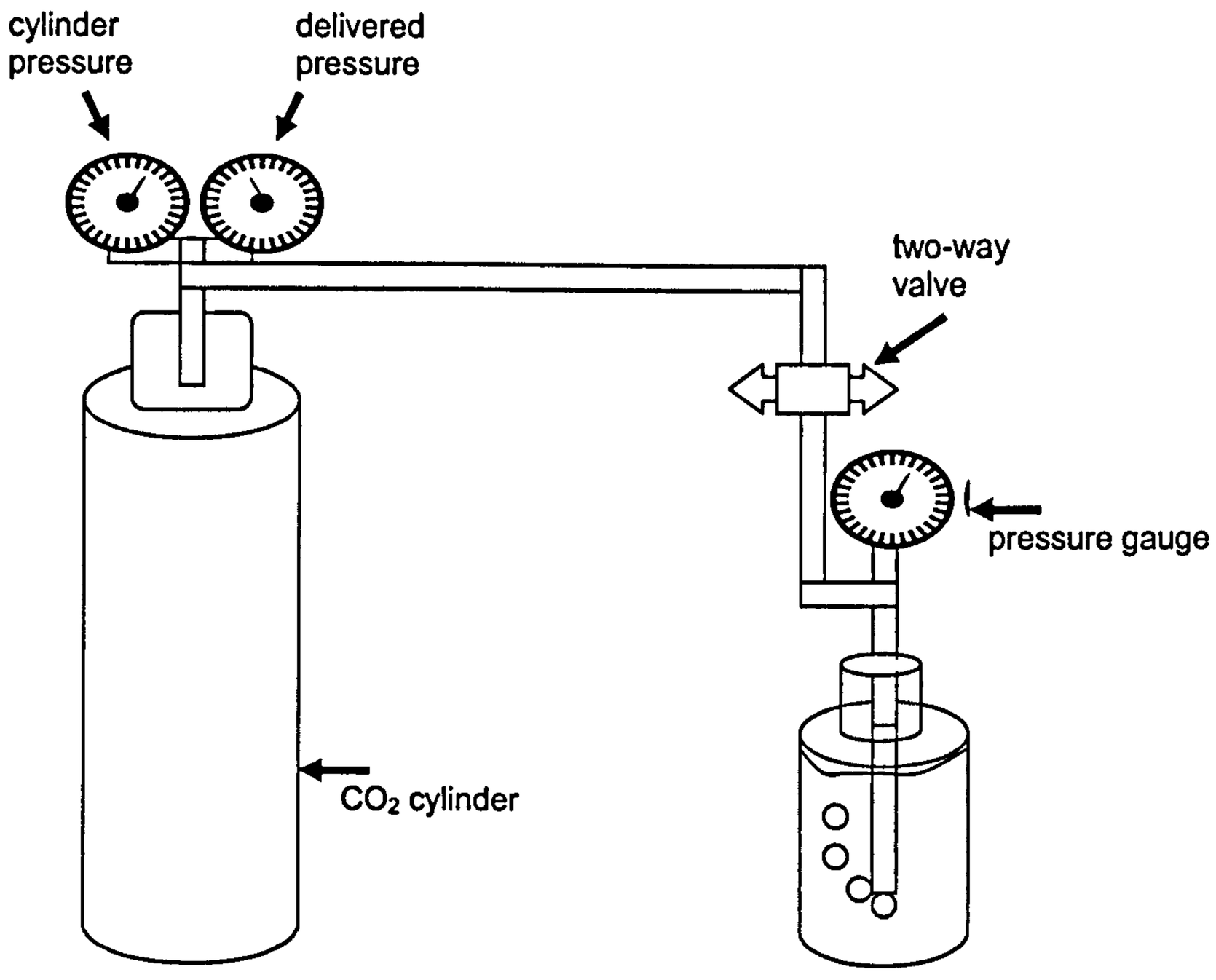


Figure 2-1: Schematic diagram of carbonating apparatus

A force-carbonation table (Table 2-1), relating temperature of sample with pressure (in psi) was used to calculate the pressure required to be applied via forced carbonation to dissolve the requisite volumes of CO_2.

Table 2-1: Carbon dioxide force-carbonation gas volume chart

		GAUGE PRESSURE IN POUNDS PER SQUARE INCH															
		0	2	4	6	8	10	12	14	16	18	20	22	24	26	28	30
	Celsius	VOLUMES of CO_2															
TEMPERATURE	0.0	1.7	1.9	2.2	2.4	2.6	2.9	3.1	3.3	3.5	3.8	4.0	4.2	4.4	4.7	4.9	5.2
	0.6	1.7	1.9	2.1	2.4	2.6	2.8	3.0	3.2	3.5	3.7	3.9	4.1	4.3	4.6	4.8	5.1
	1.1	1.6	1.9	2.1	2.3	2.5	2.7	2.9	3.2	3.4	3.6	3.8	4.1	4.3	4.5	4.7	4.9
	1.7	1.6	1.8	2.0	2.3	2.5	2.7	2.9	3.1	3.3	3.5	3.8	4.0	4.2	4.4	4.6	4.8
	2.2	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	3.3	3.5	3.7	3.9	4.1	4.3	4.5	4.7
	2.8	1.5	1.7	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.4	4.6
	3.3	1.5	1.7	1.9	2.1	2.3	2.5	2.7	2.9	3.1	3.3	3.5	3.7	3.7	4.1	4.3	4.5
	3.9	1.5	1.7	1.9	2.1	2.3	2.5	2.7	2.9	3.1	3.3	3.5	3.7	3.9	4.0	4.3	4.5
	4.4	1.5	1.6	1.8	2.0	2.2	2.4	2.6	2.8	3.0	3.2	3.4	3.6	3.8	4.0	4.2	4.3
	5.0	1.4	1.6	1.8	2.0	2.2	2.4	2.6	2.8	2.9	3.1	3.3	3.5	3.7	3.9	4.1	4.2
	5.5	1.4	1.6	1.8	2.0	2.1	2.3	2.5	2.8	2.9	3.1	3.3	3.5	3.6	3.8	3.9	4.2
	6.1	1.4	1.6	1.7	1.9	2.1	2.3	2.5	2.7	2.8	3.0	3.2	3.4	3.6	3.8	3.9	4.1
	6.7	1.3	1.5	1.7	1.9	2.1	2.2	2.4	2.6	2.8	3.0	3.1	3.3	3.5	3.7	3.9	4.0
	7.2	1.3	1.5	1.7	1.8	2.0	2.2	2.4	2.5	2.7	2.9	3.1	3.3	3.4	3.6	3.8	4.0
	7.8	1.3	1.5	1.6	1.8	2.0	2.2	2.3	2.5	2.7	2.8	3.0	3.2	3.4	3.5	3.7	3.9
	8.3	1.3	1.4	1.6	1.8	1.9	2.1	2.3	2.4	2.6	2.8	2.9	3.1	3.3	3.5	3.6	3.8
	8.9	1.2	1.4	1.6	1.7	1.9	2.1	2.2	2.4	2.6	2.7	2.9	3.1	3.2	3.4	3.6	3.7
	9.4	1.2	1.4	1.5	1.7	1.9	2.0	2.2	2.4	2.5	2.7	2.8	3.0	3.2	3.3	3.5	3.7
	10.0	1.2	1.4	1.5	1.7	1.8	2.0	2.2	2.3	2.5	2.6	2.8	2.9	3.1	3.3	3.4	3.6

Samples were carbonated by setting the delivered gas pressure to the desired level, opening the isolation switch and gently shaking the sample bottle to accelerate the dispersion of CO_2 into the sample.

Once equilibrium was achieved, as indicated by the cessation of gas entering the sample, the two-way switch was closed to isolate the sample bottle and the pressure within the bottle monitored using the second pressure gauge to ensure the correct pressure had been delivered and was maintained. Samples were carbonated to a level of 3.6vols CO_2 to be comparable to commercially available carbonated fruit flavoured beverages. The sample was then removed from the carbonating apparatus and aliquoted into glass, screw-topped vials as quickly as possible before refrigeration storage (4-6°C). Each vial was filled to the brim to reduce loss of CO_2.

Non carbonated samples were also aliquoted in the same way, and refrigerated alongside the carbonated samples to ensure parity between the two sets.

2.2.2.2. *Headspace analysis of volatile release*

Analysis of the volatile content in the headspace of samples was carried out as described in section 2.2.1.2. In addition, an initial measure of volatile concentration in the headspace from samples, both carbonated and non-carbonated, was taken immediately after samples were decanted to the sealed bottle used for headspace sampling. This measure did not reflect equilibrium between the aqueous and gaseous phases but did simulate a 'real' scenario of opening a carbonated beverage. For sensory assessment of samples, panellists would be presented with solutions in sealed vials, overfilled to exclude headspace. Therefore, for analysis of subsequent assessment of sensory attributes, the initial measure of headspace content may be pertinent. A subsequent measure of headspace volatile content was taken after 2hr sample equilibration.

2.2.3. Experiment 3: Effect of solutes on viscosity

2.2.3.1. *Sample preparation*

Samples were prepared as in section 2.2.1.1 to include varying amounts of citric acid (0g/l, 1.5g/L), lactic acid (2.6ml/L), glucose (50-150g/L) or fructose (20-60g/l). Aroma volatile concentration was constant across samples (2.5ppm each of citral and limonene.

2.2.3.2. *Instrumental measure of viscosity*

The effect of varying levels of solutes on the viscosity of the samples was investigated using a CS10 Rheometer (Bohlin, Germany) fitted with double-gap (40/50mm) stainless steel geometry. Samples (5 replicates) were prepared as described in section 2.2.3.1. Each sample (30ml) was pipetted directly into the lower cup and the upper geometry lowered to create the correct gap (150µm). All samples were analysed at a controlled shear rate between 5-50s^{-1} and constant temperature of 25°C. Sample points (20) were

recorded across the range (5-50s^{-1}) and the grand mean of 5 replicates for each sample set was calculated.

2.2.4. Statistical analysis

One way analysis of variance (ANOVA) was used to determine significant differences in mean data for Experiments 1 and 3 (sections 2.2.1 and 2.2.3). Two-way ANOVA was used to determine effects of tastants and CO_2 in Experiment 2 (section 2.2.2). Subsequent multiple comparison tests, to discriminate samples significantly different to each other, were performed using Tukey's Honestly Significant Difference test (Tukey's HSD). For all APcI-MS measured headspace data, the peak intensity of ions monitoring citral and limonene (ions 137 and 153 respectively) were analysed individually.

2.3. Results

2.3.1. Experiment 1: Effect of solutes on volatile release

The headspace of each sample was monitored for a period of 30s as the MS continually monitored m/z 153 and 137. An example chromatogram trace showing the intensity of these ions (m/z 137: limonene, m/z 153: citral) in the sampled headspace is shown in Figure 2-2. Measured levels of individual ions are shown as percentage of maximum peak height.

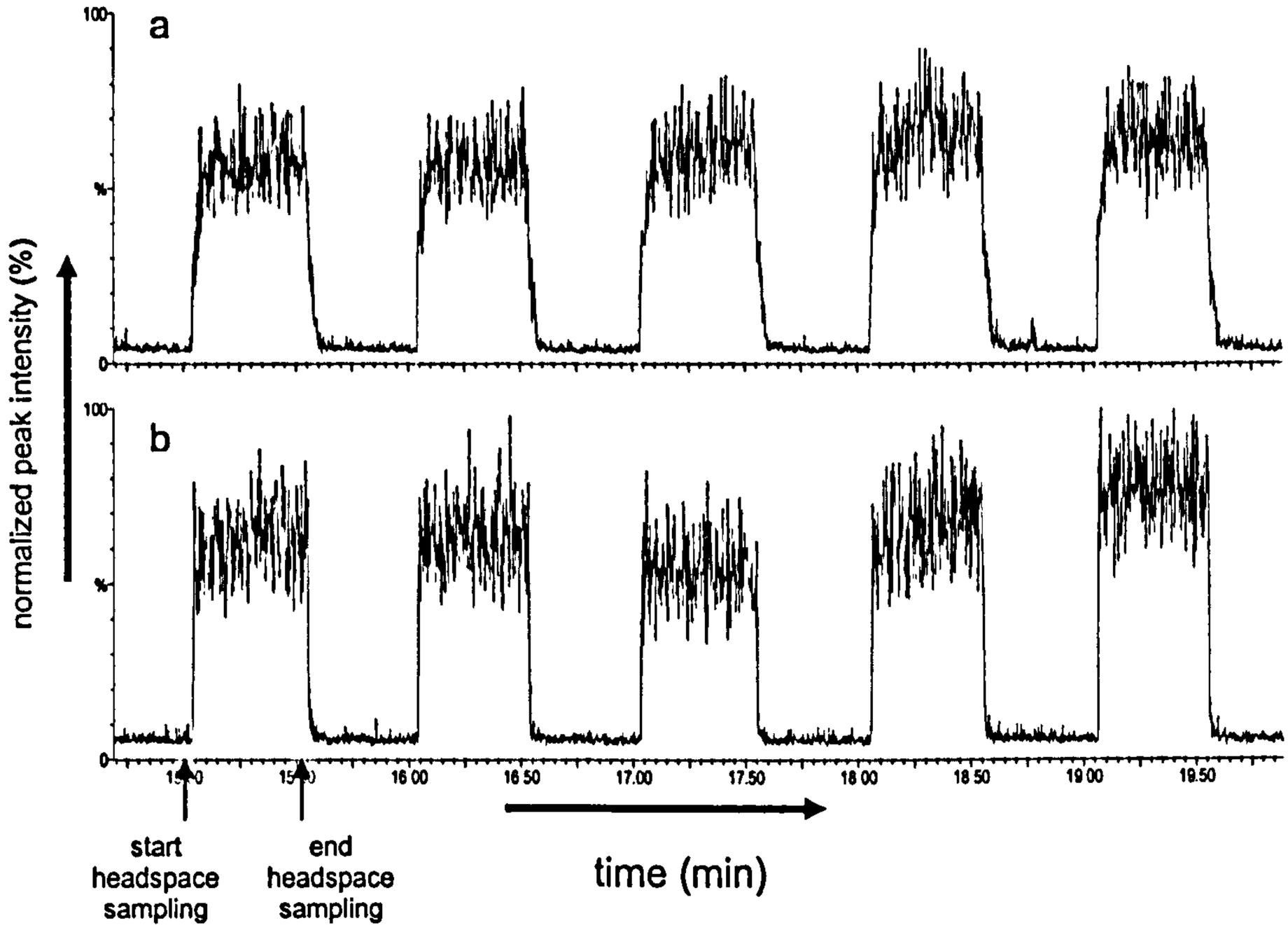
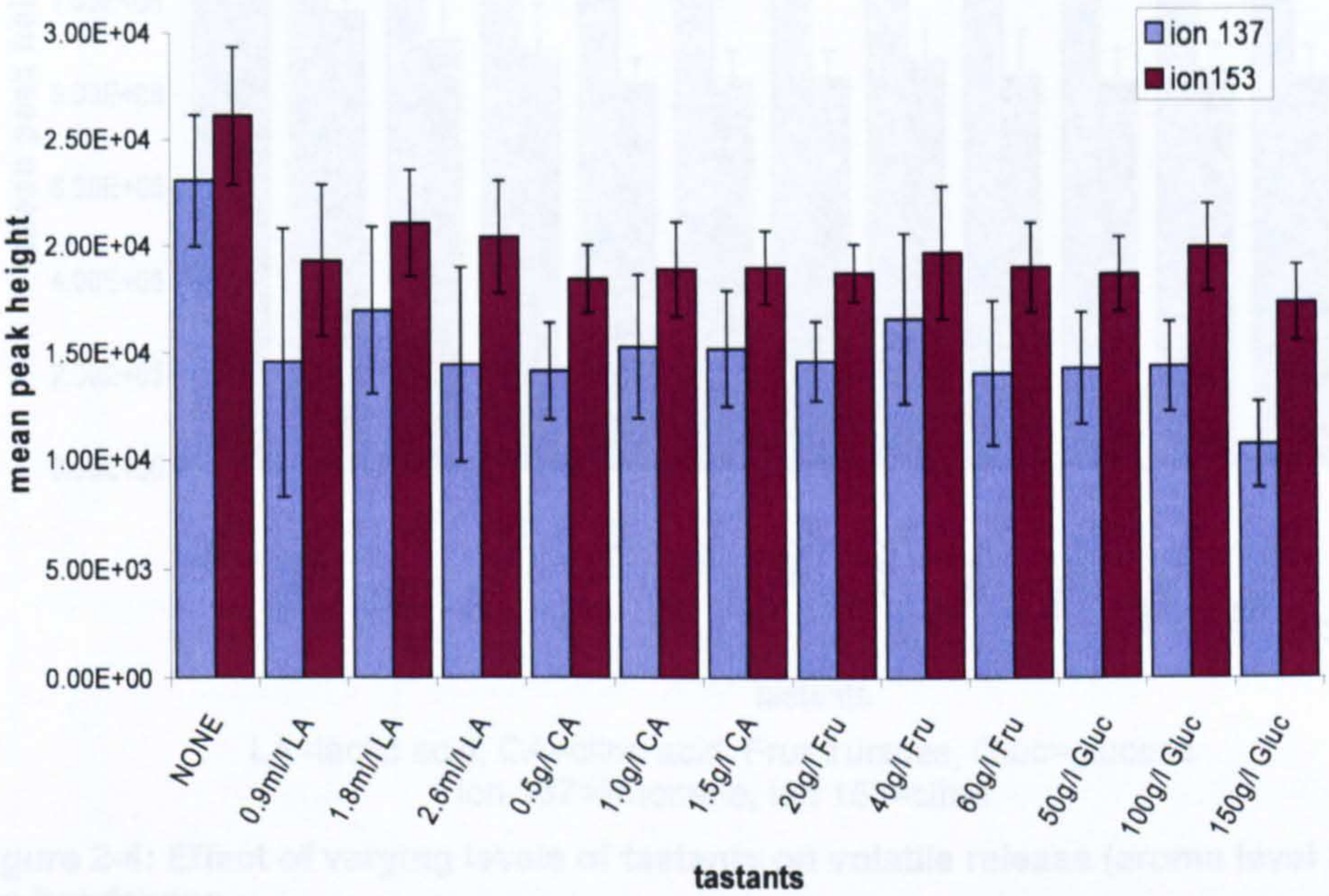


Figure 2-2: Example chromatogram of headspace volatile concentration quantification by APcI-MS for a) citral (m/z 153) and b) limonene (m/z 137) in a series of 5 samples.

Chromatograms were analysed using MassLynx software (Micromass Ltd, UK) to obtain peak height values, averaged over 30s analysis time. The mean peak height value for each experimental condition was used for subsequent data analysis. This value can be used as an indication of the number of ions formed by ionisation of the sample headspace with a mass to charge ratio of 137 or 153. The mean peak height can then be compared directly between samples to elucidate trends and differences between samples. Absolute quantification of the headspace volatile concentration could be obtained by direct injection of known amounts of the compounds under investigation into the MS. This was not required within this study as all significant changes are relative to a known reference sample; citral (2.5ppm) and limonene (2.5ppm) in the absence of solutes (and carbonation for Experiment 3).

Figure 2-3 and Figure 2-4 show the resulting mean peak height intensity of ions monitoring limonene and citral in the headspace of samples containing

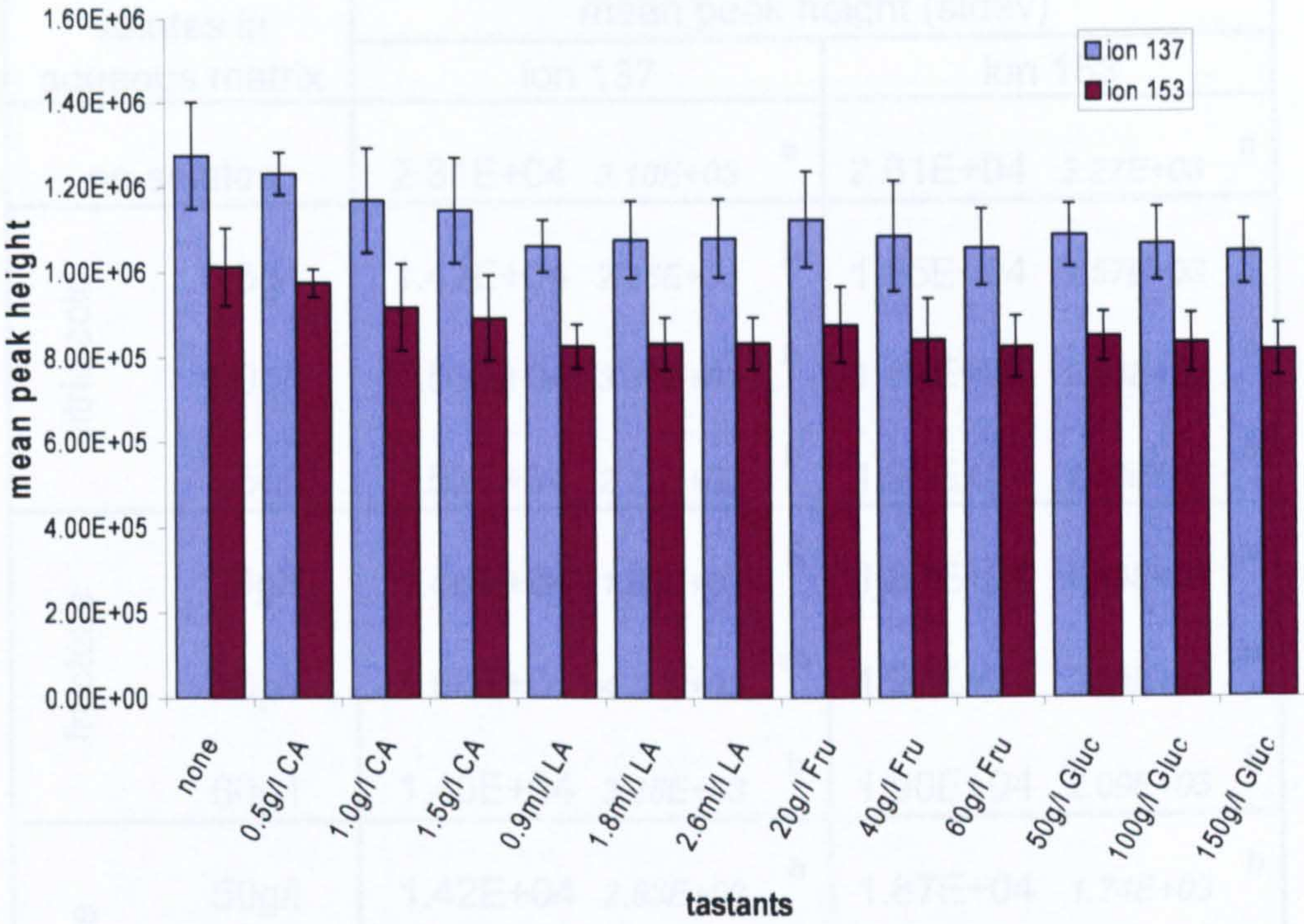
aroma levels 1 and 2 respectively (level 1; 2.5ppm each of citral and limonene, level 2; 10ppm each of citral and limonene).



Where; LA=lactic acid, CA=citric acid, Fru=fructose, Gluc=glucose, ion 137=limonene, ion 153=citral

Figure 2-3: Effect of varying levels of tastants on volatile release (aroma level 1) into the headspace.

Statistical analysis of mean peak intensity data indicated matrix composition significantly affected headspace volatile concentration (ANOVA, $p<0.05$). Multiple comparison tests (Tukey's HSD) were subsequently performed on the data. Resulting sample groupings are described in Table 2-2 and Table 2-3 together with mean and standard deviations.



LA=lactic acid, CA=citric acid, Fru=fructose, Gluc=glucose
ion 137=limonene, ion 153=citral

Figure 2-4: Effect of varying levels of tastants on volatile release (aroma level 2) into the headspace.

For samples containing aroma level 1 (2.5ppm citral and 2.5ppm limonene), multiple comparison tests determined the sample containing no solutes had a significantly higher mean peak height compared to most of the samples (Table 2-2).

Overall, results indicate a trend towards decreases in headspace volatile concentration as a result of addition of solutes. For aroma level 1, the addition of tastants produced a significant reduction in headspace volatile content (Table 2-2).

Table 2-2: Mean data and multiple comparison test groups for aroma level 1

solutes in aqueous matrix		mean peak height (stdev)			
		ion 137		ion 153	
no solutes		2.31E+04 *3.10E+03*	a	2.61E+04 *3.27E+03*	a
citric acid	0.5g/l	1.42E+04 *2.26E+03*	b	1.85E+04 *1.57E+03*	b
	1.0g/l	1.53E+04 *3.36E+03*	b	1.89E+04 *2.23E+03*	b
	1.5g/l	1.52E+04 *2.73E+03*	b	1.90E+04 *1.68E+03*	b
fructose	20g/l	1.46E+04 *1.83E+03*	b	1.88E+04 *1.36E+03*	b
	40g/l	1.66E+04 *4.00E+03*	ab	1.97E+04 *3.09E+03*	b
	60g/l	1.40E+04 *3.36E+03*	b	1.90E+04 *2.09E+03*	b
glucose	50g/l	1.42E+04 *2.63E+03*	b	1.87E+04 *1.74E+03*	b
	100g/l	1.44E+04 *2.09E+03*	b	1.99E+04 *2.05E+03*	b
	150g/l	1.07E+04 *2.00E+03*	b	1.74E+04 *1.76E+03*	b
lactic acid	0.9ml/l	1.46E+04 *6.22E+03*	b	1.93E+04 *3.54E+03*	b
	1.8ml/l	1.70E+04 *3.87E+03*	ab	2.11E+04 *2.51E+03*	ab
	2.6ml/l	1.45E+04 *4.55E+03*	b	2.05E+04 *2.62E+03*	b

Values in italics are standard deviations,

samples with the same letter within a column are not significantly different (p>0.05)

Table 2-3: Mean data and multiple comparison test groups for aroma level 2

solutes in aqueous matrix		mean peak height (stdev)			
		ion 137		ion 153	
no solutes		1.28E+06 *1.26E+05*	a	1.02E+06 *9.05E+04*	a
citric acid	0.5g/l	1.23E+06 *4.93E+04*	abc	9.77E+05 *3.22E+04*	abc
	1.0g/l	1.17E+06 *1.23E+05*	abc	9.21E+05 *1.01E+05*	abc
	1.5g/l	1.15E+06 *1.25E+05*	abc	8.95E+05 *1.00E+05*	abc
fructose	20g/l	1.13E+06 *1.13E+05*	abc	8.79E+05 *8.91E+04*	abc
	40g/l	1.09E+06 *1.31E+05*	abc	8.43E+05 *9.77E+04*	abc
	60g/l	1.06E+06 *9.07E+04*	abc	8.26E+05 *7.35E+04*	bc
glucose	50g/l	1.09E+06 *7.37E+04*	abc	8.53E+05 *5.86E+04*	bc
	100g/l	1.07E+06 *8.54E+04*	bc	8.36E+05 *7.05E+04*	bc
	150g/l	1.05E+06 *7.67E+04*	c	8.18E+05 *6.22E+04*	c
lactic acid	0.9ml/l	1.06E+06 *6.20E+04*	abc	8.28E+05 *5.22E+04*	abc
	1.8ml/l	1.08E+06 *9.02E+04*	abc	8.35E+05 *6.20E+04*	abc
	2.6ml/l	1.08E+06 *9.17E+04*	ab	8.35E+05 *6.20E+04*	ab

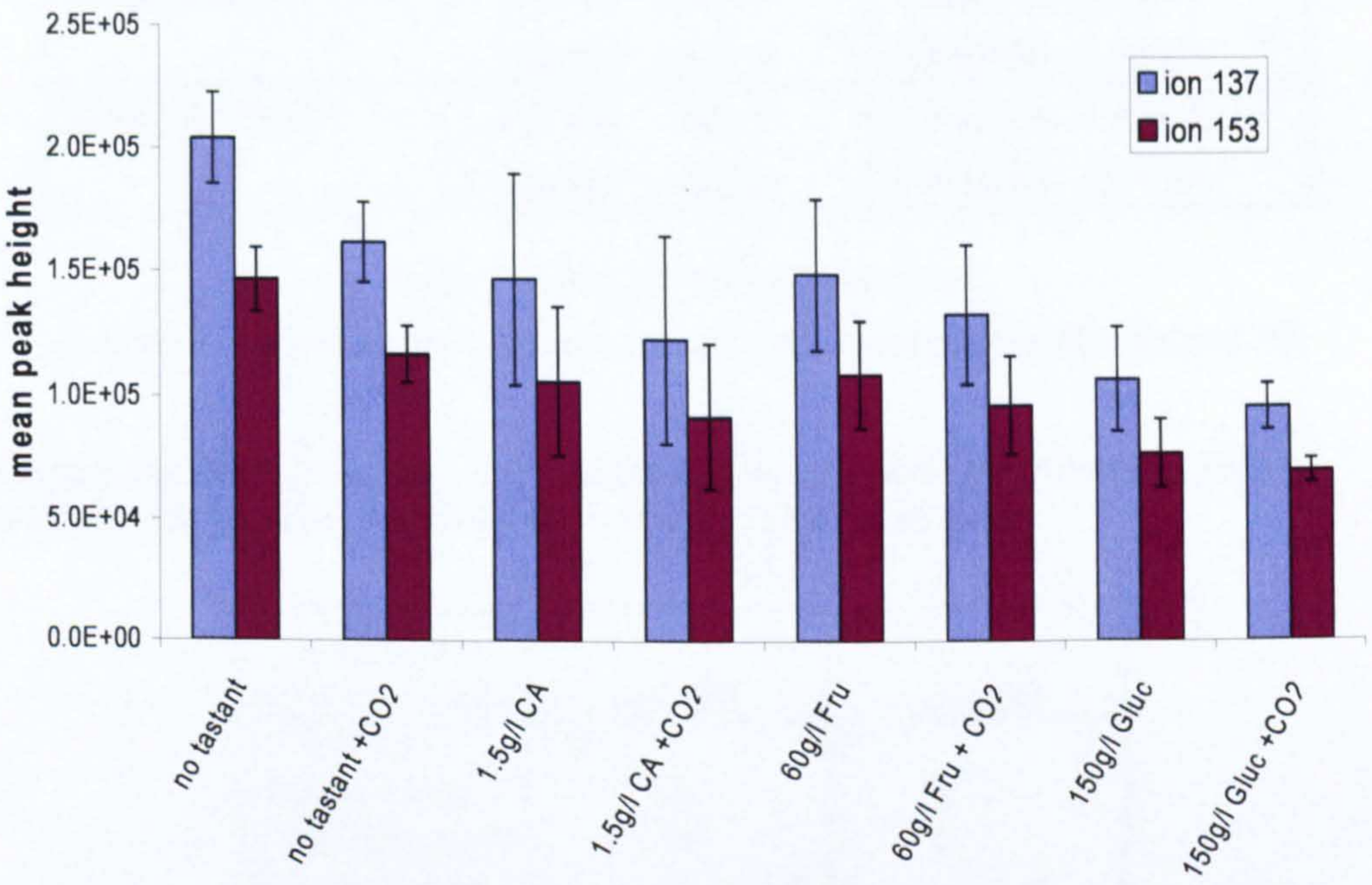
Values in italics are standard deviations,

samples with the same letter, within a column, are not significantly different (p>0.05)

A similar trend was seen for aroma level 2, but this was only statistically significant when comparing samples containing higher concentrations of sugar (ion 153; 60g/L fructose, 50-150g/L glucose, ion137;100-150g/L glucose) with those containing no solutes (Table 2-3). Examination of data from solute-containing samples (excluding aroma compound-in-water only sample) revealed no significant effect of solute type or concentration on the release of volatiles (Figure 2-3 and Figure 2-4, Table 2-2 and Table 2-3). This would indicate for each aroma level, the amount of either citral or limonene present in the headspace is not significantly affected across samples containing tastants irrespective of the concentration or type.

2.3.2. Experiment 2: Effect of carbonation on volatile release

The mean peak intensity of ions monitoring limonene (ion 137) and citral (ion 153) in the headspace of samples measured immediately after decanting from the sealed storage vial into the headspace sampling bottle are shown in Figure 2-5.



CA=citric acid, Fru=fructose, Gluc=glucose
ion 137=limonene, ion 153=citral

Figure 2-5: Effect of carbonation and tastant type on volatile release into the headspace measured immediately after decanting.

Analysis of mean peak intensity data for each sample using two-way ANOVA, with both tastant and carbonation as factors, revealed the presence of statistically significant differences between samples varying in aqueous phase matrix ($p<0.05$). The results of a subsequent multiple comparison test (Tukey's HSD) performed on the data are shown in Table 2-4, together with means and standard deviations for the sample groups.

Table 2-4: Effect of CO_2 and tastant level on headspace volatile concentration. Headspace sampled immediately after decanting.

solutes in aqueous matrix	CO_2 level	mean peak height (stdev)					
		ion 137			ion 153		
no solutes	+	2.04E+05	*1.87E+04*	a	1.47E+05	*1.30E+04*	a
	-	1.62E+05	*2.78E+04*	ab	1.17E+05	*1.96E+04*	ab
citric acid 1.5g/l	+	1.47E+05	*1.70E+04*	bc	1.06E+05	*1.41E+04*	abc
	-	1.23E+05	*1.49E+04*	bc	9.18E+04	*1.26E+04*	bc
fructose 60g/l	+	1.49E+05	*4.08E+04*	abc	1.09E+05	*3.11E+04*	abc
	-	1.32E+05	*3.95E+04*	bc	9.63E+04	*2.67E+04*	bc
glucose 150g/l	+	1.06E+05	*1.48E+04*	bc	7.69E+04	*1.14E+04*	bc
	-	9.55E+04	*2.79E+04*	c	6.99E+04	*2.21E+04*	c

Values in italics are standard deviations,

samples with the same letter, within a column, are not significantly different (p>0.05)

Table 2-5: Multiple comparison test following two-way ANOVA (factors; tastant, carbonation). Headspace sampled immediately after decanting.

solutes in aqueous matrix	ion 137	ion 153
no tastant	a	a
60g/l fructose	b	b
1.5g/l citric acid	b	b
150g/l glucose	c	c

carbonation	ion 137	ion 153
No CO_2	a	a
CO_2	b	b

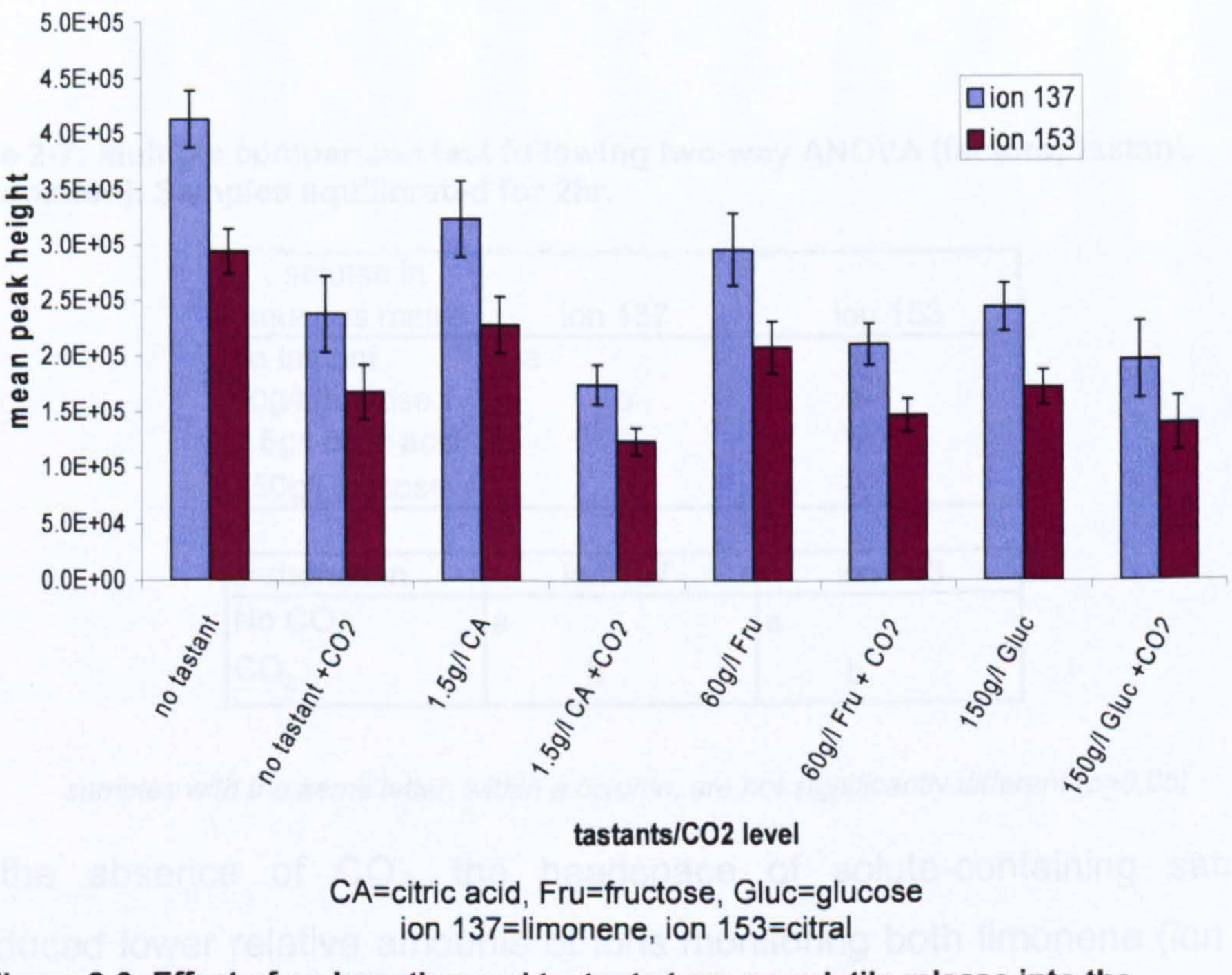
Samples with the same letter, within a column, are not significantly different (p>0.05)

Analysis of both ion 137 and 153 (limonene and citral) by two-way ANOVA, with tastant and carbonation as factors, indicated addition of tastants (1.5g/L citric acid, 60g/L fructose or 150g/L glucose) significantly reduced the volatile concentration in the measured headspace compared to samples with no tastants ($p<0.05$). Additionally, the presence of 150g/L glucose reduced the headspace volatile concentration compared to the presence of either 1.5g/L citric acid or 60g/L fructose (Table 2-5). The carbonated samples showed a

reduction in the measured headspace volatile concentration compared to non-carbonated samples (Table 2-5, $p<0.05$).

To evaluate the impact of carbonation on volatiles in a closed system at equilibrium, a subsequent measure of headspace samples was performed after 2hr. Figure 2-6 shows the mean peak intensity of both ions in sampled headspace after 2hr equilibration.

Two-way ANOVA (factors; tastant and carbonation) followed by Tukey's HSD multiple comparison test was performed on the mean data. Sample means and multiple comparison groupings are shown in Table 2-6 and groupings for each factor are shown in Table 2-7.



CA=citric acid, Fru=fructose, Gluc=glucose
ion 137=limonene, ion 153=citral

Figure 2-6: Effect of carbonation and tastants type on volatile release into the headspace after 2hr equilibration.

Table 2-6: Effect of CO_2 and tastant level on headspace volatile concentration. Samples equilibrated for 2hr.

solutes in aqueous matrix	CO_2 level	mean peak height (stdev) ion 137			ion 153		
no solutes	+	4.13E+05	*2.60E+04*	a	2.94E+05	*2.00E+04*	a
	-	2.38E+05	*3.40E+04*	d	1.68E+05	*2.43E+04*	cd
citric acid 1.5g/l	+	3.23E+05	*3.43E+04*	b	2.28E+05	*2.52E+04*	b
	-	1.75E+05	*1.76E+04*	e	1.24E+05	*1.20E+04*	e
fructose 60g/l	+	2.97E+05	*3.25E+04*	bc	2.09E+05	*2.35E+04*	bc
	-	2.12E+05	*1.89E+04*	de	1.48E+05	*1.47E+04*	de
glucose 150g/l	+	2.46E+05	*2.14E+04*	cd	1.73E+05	*1.59E+04*	cd
	-	1.99E+05	*3.49E+04*	de	1.41E+05	*2.43E+04*	de

Values in italics are standard deviations,

samples with the same letter, within a column, are not significantly different (p>0.05)

Table 2-7: Multiple comparison test following two-way ANOVA (factors; tastant, carbonation). Samples equilibrated for 2hr.

solutes in aqueous matrix	ion 137	ion 153
no tastant	a	a
60g/l fructose	b	b
1.5g/l citric acid	b	b
150g/l glucose	b	b

carbonation	ion 137	ion 153
No CO_2	a	a
CO_2	b	b

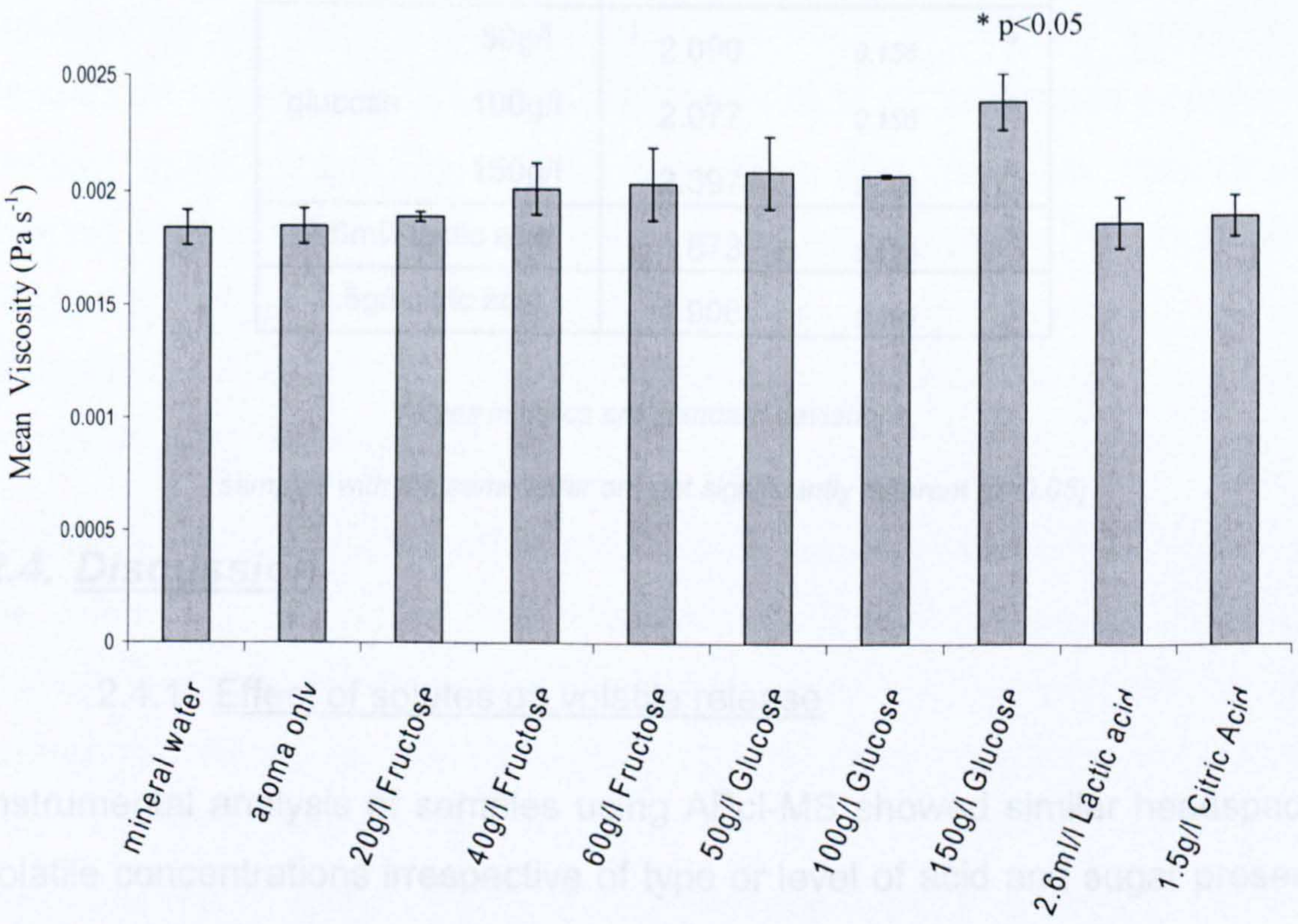
samples with the same letter, within a column, are not significantly different (p>0.05)

In the absence of CO_2, the headspace of solute-containing samples produced lower relative amounts of ions monitoring both limonene (ion 137) and citral (ion 153) when compared to headspace levels in the absence of solutes. However, after allowing samples to equilibrate for 2hr, there was no significant reduction in headspace volatile concentration between samples containing any of the three tastants. This finding is in agreement with data from Experiment 1 (Table 2-2).

Results also suggested carbonation of samples, irrespective of solute level, resulted in lower headspace levels of aroma volatiles than non-carbonated counterparts.

2.3.3. Experiment 3: Effect of solutes on viscosity

The effect of addition and concentration of citric acid, lactic acid, fructose or glucose on instrumentally measured sample viscosity was assessed using rheological protocols. The mean viscosity measurements of samples varying in composition are shown in Figure 2-7.



** indicates sample significantly different to all other samples (p<0.05)*

Figure 2-7: Effect of varying levels of tastants on instrumentally measured viscosity.

Results of ANOVA performed on mean viscosity data showed the presence of significant differences within the sample set and subsequent Tukey's multiple comparisons revealed the sample containing 150g/L glucose had a significantly higher viscosity compared to all other samples (Table 2-8). This equates to an increase in viscosity of approximately 0.4mPa.s. No significant

differences in viscosity were seen between other samples irrespective of type or level of tastant.

Table 2-8: Mean viscosity for samples varying in solute content

solutes in aqueous matrix		mean viscosity (mPa.s)		
mineral water		1.843	*0.077*	a
no solutes		1.852	*0.077*	a
fructose	20g/l	1.893	*0.112*	a
	40g/l	2.013	*0.021*	a
	60g/l	2.037	*0.112*	a
glucose	50g/l	2.090	*0.158*	a
	100g/l	2.077	*0.156*	a
	150g/l	2.397	*0.006*	b
2.6ml/l lactic acid		1.873	*0.120*	a
1.5g/l citric acid		1.906	*0.090*	a

Values in italics are standard deviations,

samples with the same letter are not significantly different (p>0.05)

2.4. Discussion

2.4.1. Effect of solutes on volatile release

Instrumental analysis of samples using APcI-MS showed similar headspace volatile concentrations irrespective of type or level of acid and sugar present when analysed across each aroma level. Nonetheless, in Experiment 1, the addition of tastants (acid or sugar) resulted in an overall decrease in headspace volatile concentration when compared to volatiles-in-water only samples. This was supported by findings in Experiment 2, where headspace measures of samples at equilibrium were lowered in the presence of tastants irrespective of carbonation level (Figure 2-6 and Table 2-7).

These findings are in agreement with Massaldi and King (1973) who reported findings of decreases in activity coefficient of limonene on addition of sucrose

at levels (10 and 20%) relevant to those in the present study (2-6% fructose, 5-15% glucose). A decrease in activity coefficient signifies there are fewer interactions between solute and solvent yet more between the volatile and solute. This would suggest, as the activity coefficient of limonene decreases (on addition of sucrose), a concurrent decrease in headspace concentration of limonene would result.

Other groups (Friel *et al.* 2000; Hansson *et al.* 2001; Rabe *et al.* 2003) have shown changes in volatile release profiles may follow solute concentration dependant trends. However, the levels of solutes examined (up to 65% sucrose) far exceed the range used in this study which may explain why headspace volatile levels showed minimal solute concentration dependant trends (Figure 2-3 and Figure 2-4). Acids have also previously been shown to affect volatile release in a soft drink-related model system (Hansson *et al.* 2001) and fruit pulp system (Marsh *et al.* 2006). This is supported by reports of decreases in activity coefficients of volatiles on addition of acid to aqueous aroma solutions (Voilley *et al.* 1977).

In spite of the finding that addition of solutes results in some modification of the release profile of the two volatiles included in the model beverage system, it is not fully clear what impact this would have on perception. For retronasal delivery of volatiles to the nasal odour receptors, the aroma molecules must partition from the matrix which will be additionally diluted by saliva. Other factors also influence release such as composition of saliva, oral temperature, mastication and binding of aroma volatiles to the mucosa (Taylor 1998). Direct measurement of volatile concentration in exhaled breath was not possible due to volatile levels being below instrumental limits of detection. Therefore the effect on volatile release of these factors cannot be accounted for.

Few studies have combined examination of flavour release, measured instrumentally, and flavour perception, assessed by sensory evaluation. King et al (2006) and Asquith and Swaine (2002) explored the effects of common

sweeteners (sucrose, ace-K, aspartame and high fructose corn syrup respectively) on both flavour release and perception in beverage systems. Whilst reporting evidence of solute modification of volatile release, they concluded that sweetener level had a greater impact on flavour perception than effects on volatile release profiles would predict.

These findings would indicate that differences in perceived flavour intensity, between samples with a solute content within the range of tastants examined, would not be expected to be a direct result of alterations in the physical release of volatile from the beverage matrix.

2.4.2. Effect of carbonation on volatile release

In Experiment 2, the effect of carbonation on volatile release was examined. Whilst the headspace measures obtained immediately after decanting do not relate to equilibrium partitioning of the volatiles between the aqueous and gaseous states, they directly replicate the scenario on first opening a carbonated beverage. Sensory evaluation was to be performed immediately after opening samples in sealed vials without the presence of gaseous headspace (samples fully occupy vial). Consequently, it was appropriate to mimic this situation in volatile release measurements.

Interestingly, measurement of headspace immediately after decanting and after equilibrating for 2hr suggested carbonation may influence volatile release. Data described in Figure 2-5 and Figure 2-6 and results of multiple comparison tests (Table 2-5 and Table 2-7) indicated relative amounts of both monitored ions were decreased in samples which had been previously carbonated. This may reflect decreased activity coefficients of citral and limonene suggesting interactions between dissolved CO_2 and the two volatiles.

Alternatively, it is feasible that opening the sealed vial, to decant samples for headspace monitoring, released the CO_2 dissolved within the aqueous phase (as the pressure is removed) and this acted in the same way as introducing a

gas flow into the sample. As a consequence, aroma volatiles may have been carried with the gas, into the surrounding air at a greater rate than in non-carbonated samples.

The modification of volatile release by carbonation may be an influencing factor on flavour perception and particularly the temporal flavour perception profile during consumption of a commercial carbonated beverage.

2.4.3. Effect of solutes on viscosity

On the whole, addition of tastants did not effect the viscosity of the samples, with the exception of the highest level of glucose studied (150g/L) which resulted in a significant increase in viscosity ($p<0.05$, Figure 2-7).

The concentration range of glucose was larger than fructose to allow comparison of the two monosaccharides at levels determined to be perceptually equi sweet. Fructose has a Relative Sweetness Index (RSI) of 0.7 whilst glucose has a RSI of 1.4 (RSI of sucrose is 1). Therefore, almost double the amount of glucose would be required to confer a perception of sweetness equal to that of fructose. This ultimately means the amount of solute is greater. Viscosity data suggests this did not appear to significantly modify instrumental viscosity at the lower sugar ranges and only at the highest limit of glucose range did the amount of solute impact on viscosity.

Previous studies have shown that changes in textural attributes of solutions such as in viscosity, can result in perceptual differences in other modalities e.g taste and aroma (Burns *et al.* 1986; Walker 2000; Cook *et al.* 2005). Moskowitz and Arabie (1970) found that increasing viscosity of a solution leads to a decrease in sourness perception (although pH remains unaffected) which was supported by Walker and Prescott (2000) who also found a suppression of flavour intensity by increasing viscosity. Variable findings have been demonstrated regarding effects on sweetness perception but most agree that suppression occurs with increasing thickness (Burns *et al.* 1986). However, these previous studies generally used specific thickeners to

change viscosity such as xanthan, hydroxypropylcellulose, and carboxymethylcellulose, which may have direct effects on other sensory properties.

In these studies, increased viscosity is only seen with the largest glucose concentration used. Instrumental measures of headspace volatiles using APCI-MS (section 3.3) suggest that even this highest concentration of glucose used does not significantly affect the volatile release profile (Figure 5). This is in agreement with reports suggesting at low sugar ranges (<20%sucrose), effects on partition coefficients are more influential than viscosity effects on mass transfer (Rabe *et al.* 2003). Hence, it is doubtful that the small viscosity differences across the model beverage system will cause a significant impact on perception of flavour as a direct result of modification of volatile diffusion within the aqueous matrix.

Despite statistical significance, the increase in instrumentally measured viscosity of the 150g/L glucose sample is small (approximately 0.4mPa.s) and it could be debated as to whether this would produce a perceivable textural difference in mouth. Interestingly, a recent study by Kappes et al (2006) implies a mouthfeel difference may be detectable between diet and regular carbonated beverages varying in instrumental viscosity by 0.527mPa.s but this was very much dependant on individuals viscosity thresholds. This suggests the viscosity increase identified on addition of 150g/l glucose is unlikely to be identifiable 'in-mouth' by assessors and therefore would not be expected to influence on sensory perception.

2.5. Conclusions and summary

Physico-chemical effects of altering level and type of solute were examined, using APCI-MS to investigate flavour release and rheometry to study viscosity.

The presence of solutes within the aqueous phase of the model beverage samples resulted in a reduction in relative volatile headspace concentration compared to non-solute containing samples. Despite this, variation in type or amount of tastant present appeared to have no further effect on volatile release over the ranges studied in this system.

Analysis of the carbonated samples revealed carbonation had a significant effect on volatile content of headspace sampled both immediately after decanting samples from sealed vials and after 2hr equilibration. This may be a direct result of CO_2 release from the aqueous phase causing a gas flow through the matrix and flushing volatiles out into the surrounding atmosphere at a greater rate then non-carbonated samples, prior to decanting into headspace sampling vessels. This reduction in volatile release may have implications on sensory perception of flavour in carbonated beverages.

Analysis of viscosity of samples varying in solute type and level showed a small but significant increase in instrumentally determined viscosity at the highest glucose level examined (150g/L). Within the solute ranges examined no other differences in viscosity were identified. This increase in viscosity did not appear to influence volatile diffusion through the aqueous matrix, as indicated by headspace measurement of volatiles remaining consistent across levels of solutes.

From these studies, it would seem that variation in the tastant composition of beverages, within the concentration range constraints, would result in minimal modification of aroma volatile release and minor viscosity changes. The findings provide evidence of only limited physicochemical interactions occurring within this model system which may influence the sensory perception of such products.

3. Taste-aroma interactions

3.1. *Introduction*

The concept of multimodal sensory perception encompasses integration of sensory input from each of our sensory modalities (taste, smell, texture and mouthfeel, sight, sound) and, the influence of interactions occurring within and across these modalities, as previously discussed in Chapter 1.

Flavour perception is commonly considered to be primarily determined by the gustatory, olfactory and trigeminal (somatosensory input, such as tactile, thermal, irritation) systems, but visual and auditory signals also contribute to the perception of flavour (Noble 1996; Duran *et al.* 1999; Zampini *et al.* 2004; Zampini *et al.* 2005). Interactions, both within and between modalities may impact on perception and are, therefore, an important influence on the overall perception during gustation. Interactions which impact on the perception of foods are commonly seen between the gustatory and olfactory systems.

The occurrence of physico-chemical interactions between volatile and non-volatile components of a food may modify odour, taste and textural sensorial responses before neural processing. These have previously been investigated in the model citrus-style beverage system (Chapter 2). However, interactions may also result from physiological, cognitive or psychological effects and influence perception without directly modifying physical or chemical characteristics of a food (Noble 1996; Prescott 1999).

Within the model beverage system both aroma volatiles (citral and limonene) and tastants (citric or lactic acid, glucose or fructose) are varied to enable assessment of their impact on perception. In order to understand the implications of varying the composition of the system and any inter-modal or intra-modal interactions on sensory perception, published literature is

reviewed from both intra-modal (taste-taste) and cross-modal (taste and aroma) interactions and the resultant perceptual effects.

3.1.1. Taste-taste interactions

It has been widely established that taste perception changes when multiple taste stimuli are presented together rather than in isolation. Many research groups have reported findings from a variety of tastant mixtures, including different tastant sub-types and concentrations, but all provide some evidence of taste-taste interactions (Pangborn 1961; Pangborn *et al.* 1964; Moskowit.Hr 1972; Curtis *et al.* 1984; Wada *et al.* 1985; Frank *et al.* 1987; Calvino *et al.* 1990; McBride *et al.* 1990; Schifferstein *et al.* 1991; Frank *et al.* 1993; Frijters *et al.* 1994; Breslin 1996; Walters *et al.* 1996; Keast *et al.* 2003).

Much work has focussed on perceptual interactions between sweet and sour tastes. Pioneering work by Pangborn *et al* (Pangborn 1961; Pangborn 1963; Pangborn *et al.* 1964) examined the relationship between two common tastants, sucrose and citric acid. These authors found citric acid suppressed the intensity of sweetness due to sucrose and, inversely, that sucrose was able to suppress sourness due to citric acid. This work has been repeated and expanded by subsequent investigations and, despite some conflicting reports (Kamen *et al.* 1961; Curtis *et al.* 1984) , most studies agree that at suprathreshold levels, sweetness and sourness mutually suppress one another (Pangborn 1961; Frank *et al.* 1986; Frank *et al.* 1987; McBride *et al.* 1987; McBride *et al.* 1990; Schifferstein *et al.* 1990; Bonnans *et al.* 1993). There is some evidence suggesting that this suppression is concentration dependant but this finding mainly relates to subthreshold levels of tastants when enhancement may occur (Kamen *et al.* 1961).

Both Schifferstein *et al* (1991) and Bonnans and Noble (1993) provided evidence to suggest sugars and sweeteners (sucrose, fructose, aspartame, sorbitol, saccharin) show equal suppression of citric acid at perceptually equi-

sweet levels. Conversely, a study by Savant and McDaniel (2004) found that glucose caused an initial enhancement of the sourness of both citric and lactic acids followed by only marginal suppression. In contrast, the same group reported perceptually equi-sweet levels of both fructose and sucrose suppressed sourness of citric and lactic acids. Savant *et al* (2004) used ranges of acids and sugars common in commercial beverages and examined equi-molar, equi-weight and equi-sweet sugar levels. The authors concluded that sourness suppression was not mediated by molarity or weight, but the perceived intensity of the sugars. The finding of differences in ability to suppress sourness between the three sugars was proposed to indicate a different receptor or receptor mechanism for glucose, which in turn would suggest a peripheral component to suppression.

Whether it is suppression of sweetness by acid or suppression of sourness by sugar that has the greatest influence in binary mixtures, would appear to be dependant on tastant ranges investigated. Bonnans and Noble (1993) reported a larger suppression of sweetness by increasing acid concentration (0.75-2.25g acid/L) than suppression of sourness by increasing sugar level (80-120g sucrose/L). Schifferstein and Frijters (1990) examined a wider range of sucrose concentrations (0-340g/L) and found minimal suppression of sweetness by increasing citric acid (0-2g/L) with a greater degree of sourness suppression by sucrose. These authors also reported that sweetness suppression was dependant only on the level of citric acid, whilst sourness suppression was dependant on both acid and sugar levels.

These reported findings suggest a complexity to the relationship between sugars and acids dependant, to some extent, on tastant type and concentration. The most common observation is that binary combinations of suprathreshold levels of acid and sugar in aqueous solutions display mutual suppression of the individual taste qualities.

3.1.2. Taste-aroma interactions

Previous research investigating the relationship between olfactory and gustatory stimuli have shown effects of tastants on the perception of flavour intensity and of flavour compounds on the perception of taste intensity (Bonnans *et al.* 1993; Frank *et al.* 1993; Nahon *et al.* 1996; Hansson *et al.* 2001; Djordjevic *et al.* 2004; King *et al.* 2006). However, there has been much debate as to the nature of these interactions and whether flavour is a result of analytic (addition of separate dimensions) or synthetic (components indistinguishably merged) perception.

Early work by Murphy *et al* (Murphy *et al.* 1977; Murphy *et al.* 1980) produced data which suggests the gustatory and olfactory systems function independently and that the overall flavour intensity is a result of simple addition of intensities of the component parts of the mixture. Although enhancement of taste intensity in the presence of aroma compounds (both ethyl butyrate and citral) was reported, this was attributed to confusion between taste and smell. This view was supported by Frank *et al* (Frank *et al.* 1988; Frank *et al.* 1989; Frank *et al.* 1993; Frank 2003), who found that both taste-taste and taste-odour integration was dependant to some extent on the number of response options presented. For example, Frank *et al* (1993) found that when strawberry flavoured, sweetened solutions were rated only for sweetness, flavour enhanced sweet ratings, but when the solutions were rated for a number of attributes (sweetness, sourness and fruitiness) this enhancement was lost. This may be an example of the 'dumping' effect described by Lawless and Clark (1992), whereby ratings are placed into inappropriate scales when response alternatives are limited.

More recent literature, however, has provided compelling evidence that gustation and olfaction are not fully independent, and that perceptual interactions may occur at a subconscious level. This phenomenon was convincingly demonstrated by Dalton *et al* (2000), who showed a decrease in threshold detection level of an aroma when presented in combination with a

subthreshold level of a tastant. These authors used a congruent pairing of aroma and tastant (benzaldehyde and saccharin) and were able to demonstrate a significant and reliable (9 out of 10 assessors) increase in sensitivity to orthonasally delivered benzaldehyde in the presence of sub-threshold concentrations of saccharin held in the mouth. Congruency has been defined as 'the extent to which two stimuli are appropriate for combination in a food product' (Schifferstein *et al.* 1996). This 'appropriateness' appears to influence taste-aroma interactions and indeed, Dalton (2000) reported that the increase in sensitivity to benzaldehyde was not reproduced when either water alone or sub-threshold concentrations of MSG were held in mouth.

This finding of subthreshold taste-aroma interactions was supported by Pfeiffer *et al* (2005), who reproduced Dalton's experiment with a different panel, and expanded it to conclude that aroma-taste interaction occurred even when the olfactory stimulus was delivered retronasally providing residual tastant was still present within the mouth.

Recently, Labbe et al (2007), demonstrated that subthreshold olfactory stimulation can enhance sweetness. Using a continuous liquid flow system (based on the Dynataste system of Hort and Hollowood (2004)), Labbe's group established subthreshold levels of ethyl butyrate significantly increased the perceived sweetness of a sucrose solution. Despite using the same paradigm to examine a different odorant, maltol, results were inconsistent for sweetness enhancement with this volatile. The authors attribute this to a decrease in congruency of the maltol-sweetness pairing compared to the ethyl butyrate-sweetness pairing.

These findings suggest that not only do taste-aroma interactions occur, which impact on the perception of mixture components and that these may be congruency dependant, but that this interaction is occurring as a result of convergence of gustatory and olfactory stimuli at a cognitive level. Neuroimaging studies (Small *et al.* 1997; Kadohisa *et al.* 2005; Rolls 2005;

Small *et al.* 2005) of olfaction, gustation and flavour have identified a number of overlapping areas responsive to these stimuli, and single cell recordings in mammalian studies have shown the existence of neurons responsive to both taste and smell (Kadohisa *et al* 2005). Intriguingly, some brain regions show a non-additive response to input from taste and olfaction; these areas are activated by combined presentation of odour and taste to a greater degree than the sum of each stimuli presented independently (de Araujo *et al.* 2003). These studies provide persuasive evidence that processing of gustatory and olfactory information are not wholly independent, and that they may influence perception of one another via interactions at chemical, physical and/or neuronal levels.

As previously mentioned, a number of groups have observed perceptual effects on combining tastants and aromas, most studies suggest an enhancement of flavour perception by sweetness and sourness (McBride *et al.* 1987; Bonnans *et al.* 1993; Pfeiffer *et al.* 2006), but further evidence for the importance of congruency, i.e. how harmonious or 'expected' the taste-aroma pairing is, is evident in many studies (Frank *et al.* 1988; Schifferstein 1995; Dalton *et al.* 2000; Diamond *et al.* 2005).

Enhancement appears to be reciprocal if the pairing is congruent, i.e.addition of odours can enhance the perception of taste and addition of taste can enhance the perception of odours. For example, 'sweet-smelling' aromas increase perceived sweetness and suppress sourness (Frank *et al.* 1989; Stevenson *et al.* 1999; Djordjevic *et al.* 2004), and a limited effect can be seen with imagined odours (Djordjevic *et al.* 2004) suggesting a centrally mediated integration. The latter study provided further evidence for the theory that the interaction between tastant and aroma is perceptual, and occurs at a neural or cognitive level rather than at a physico-chemical level. This work is supported by the previous studies by Dalton *et al* (2000), and subsequent studies by Pfeiffer *et al* (2005) and Labbe *et a*l (2007), which used sub-threshold levels of tastant/aroma.

Most of these studies concentrate on sucrose to provide sweetness and citric acid to provide sourness, although a range of type and complexity of aroma compounds/blends have been investigated. Few studies have examined the impact of tastant addition on sensory perception alongside examination of tastant effects on flavour volatile release profiles via physico-chemical interactions.

To expand current knowledge of taste-aroma interactions, a model beverage system was created to investigate the effect of varying levels of acids and sugars, on both perception and flavour release. A simple mix of 2 aroma volatiles (limonene and citral) was chosen to create a citrus-style flavour. Two naturally occurring sugars: glucose and fructose, frequently found in commercial beverages both in combination (glucose-fructose syrup) and alone (high levels of glucose are present in sports/energy drinks), and two naturally occurring acids; citric (commonly found in citrus fruits) and lactic (commonly found in dairy products), were included as design factors. The concentration of these was varied within the ranges normally found in commercial beverages, enabling the construction of a model design space Within this design space, vertices (design factors) were defined as sugar type and concentration, acid type and concentration and aroma volatile concentration.

The objectives of this study were to determine the impact of varying concentrations of each design factor on flavour perception and, ultimately, to generate an equation to predict flavour perception for any level of aroma, acid and sugar within this model system. This equation would then allow identification of multimodal (taste-aroma) interactions occurring within this model beverage system. Extending the study to include evaluation of sweetness and sourness perception, allows assessment of taste-taste interactions alongside reciprocal aroma-taste interactions.

These findings, in combination with the physical flavour release and viscosity results described in Chapter 2, will elucidate the relationship between taste

and aroma, the physical and psychological interactions between the two modalities and the impact of these interactions on sensory perception within the model beverage.

3.2. Materials and Methods

3.2.1. Sensory panel

A total of 12 assessors (3 male, 9 females, aged between 43-68yrs) from the University of Nottingham (UoN) external sensory panel, were invited to take part in the study after completing appropriate screening tests involving the samples under investigation. All assessors had been members of the UoN sensory panel for between 5 and 9 years and had previous experience of a wide range of sensory evaluation procedures. Panellists also had previous experience of deconstructing flavour into its component parts in a range of matrices, from simple solutions to complex food systems.

Screening tests included identification and ranking of solutions varying in concentration of each tastant or aroma volatile. Following selection, the assessors participated in several training sessions in the technique of Magnitude Estimation (BS ISO 11056:1999) before commencing the study.

3.2.2. Experimental model design space

D-optimal designs (created in Design Expert software, Stat-Ease Inc, Minneapolis) were constructed using glucose (0-150g/l) or fructose (0-64g/l), and citric (0-1.5g/l) or lactic acid (0-2.63ml/l) as numerical factors and aroma volatile level (2.5ppm or 10ppm each citral and limonene, aroma level 1 and 2 respectively) as a categorical factor. Sugar and acid levels were chosen to be within the ranges found in commercially available beverages, and these ranges have previously been reported to be perceptually equi-sweet and equi-sour respectively (Savant and McDaniel, 2004). A schematic outline of this design is shown in Figure 3-1.

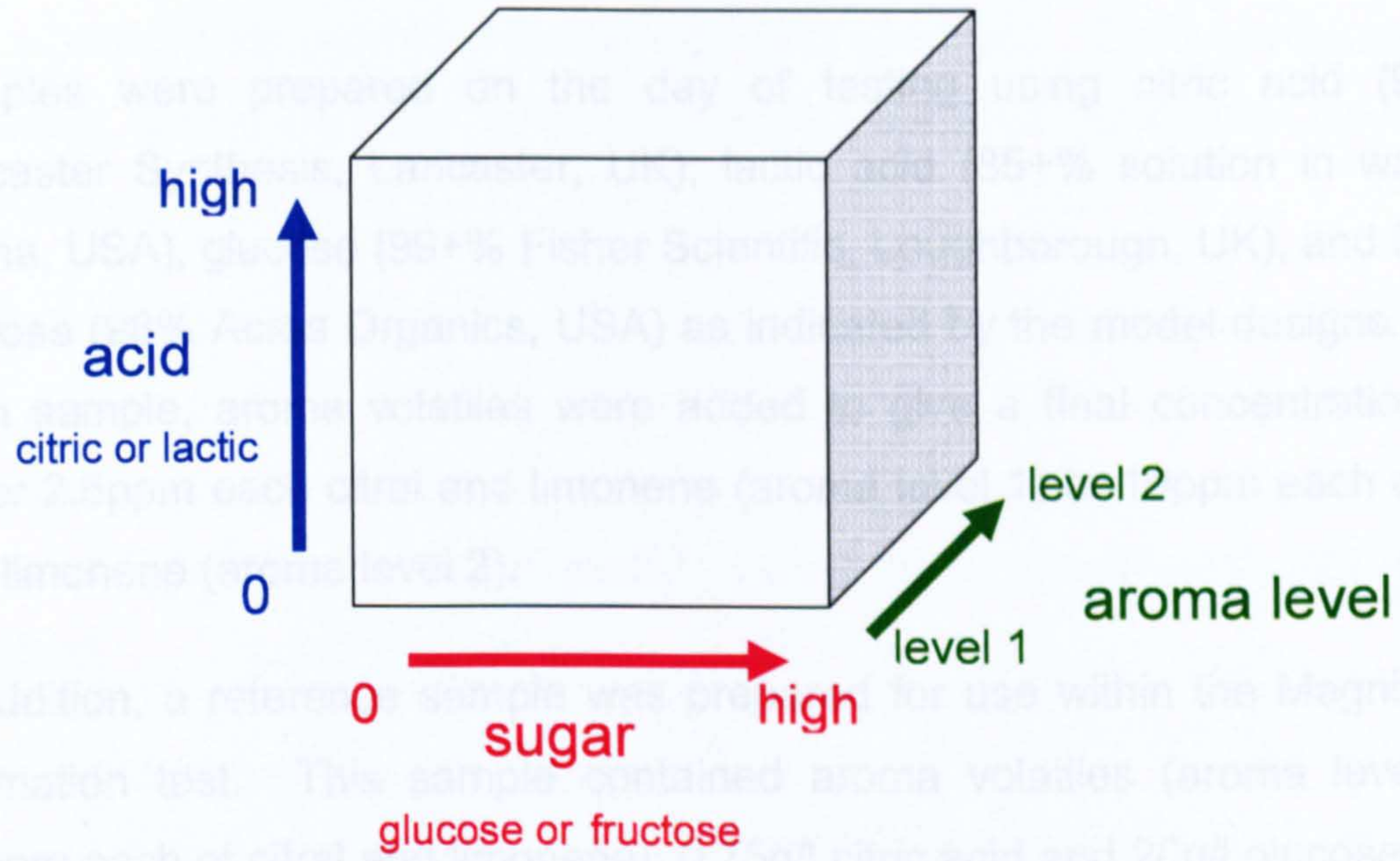


Figure 3-1: Schematic representation of model design space

Four models were generated and are described in Table 1. 16 samples from within the design space of each model (including 3 replicate points) were used for evaluation of perception.

Table 3-1: Tastant pairings in Models 1-4

	Sugar	Acid	Aroma (citral/limonene blend)
MODEL 1	Glucose (0-150g/L)	Lactic (0-2.63ml/L)	2.5ppm and 10ppm
MODEL 2	Fructose (0-64g/L)	Lactic (0-2.63ml/L)	2.5ppm and 10ppm
MODEL 3	Glucose (0-150g/L)	Citric (0-1.5g/L)	2.5ppm and 10ppm
MODEL 4	Fructose (0-64g/L)	Citric (0-1.5g/L)	2.5ppm and 10ppm

3.2.3. Sample preparation and presentation

A citrus-like aroma was created using two volatiles, limonene and citral (Aldrich, Dorset, UK). Two flavour levels were created using a blend of equal amounts of both volatiles: either 2.5ppm or 10ppm of each aroma compound.

The two levels of aroma volatiles, dissolved in mineral water (Brecon Carreg, UK) resulted in solutions perceivably different in citrus-like flavour intensity.

Samples were prepared on the day of testing using citric acid (99% Lancaster Synthesis, Lancaster, UK), lactic acid (85+% solution in water, Sigma, USA), glucose (99+% Fisher Scientific, Loughborough, UK), and D(-)-fructose (98% Acros Organics, USA) as indicated by the model designs. To each sample, aroma volatiles were added to give a final concentration of either 2.5ppm each citral and limonene (aroma level 1) or 10ppm each citral and limonene (aroma level 2).

In addition, a reference sample was prepared for use within the Magnitude Estimation test. This sample contained aroma volatiles (aroma level 1, 2.5ppm each of citral and limonene), 0.75g/l citric acid and 20g/l glucose and was consistent for all model designs. All samples were subsequently mixed on a roller bed for a minimum of 1hour to ensure all components were fully dissolved and dispersed. Samples were stored and used at ambient room temperature.

Samples (7.5ml) were presented in identical semi-translucent plastic pots (30ml) with lids, each labelled with a randomly generated 3 digit code, in a randomised, balanced order across the panellists. Samples were presented monadically, in sets of 3, with breaks of 15 minutes between sets. Assessors were instructed to consume the whole sample, make their assessment and palate cleanse using cracker and water between samples. A minimum of 1minute was allowed before presenting the next sample in the set of 3 to ensure no carry-over effects.

For each model, a total of 18 samples (16 from design space + 2 blind reference samples) were evaluated per session by each assessor, with duplicate samples assessed in a subsequent, separate session. The reference sample was included within each session to function as an internal control to allow assessment of panel performance.

Samples from all models were assessed for perceived flavour intensity and samples from Models 3 (glucose/citric acid) and 4 (fructose/citric acid) were also assessed for perceived sweetness and sourness. Each attribute was evaluated during separate sessions bringing the total number of sessions each assessor attended to 16.

In two subsequent sessions, an independent set of samples (validation set) were chosen from the design space of Models 3 and 4. These samples were evaluated for perceived flavour, sourness and sweetness attributes, in duplicate, to enable predictive models generated by the original data to be validated.

3.2.4. Sensory panel training

The technique of Magnitude Estimation with a fixed reference (BS ISO 11056:1999) was used to evaluate the design space samples. This is a ratio-scaling method based on Stevens' Law, so the resultant data have ratio-properties, similar to the standard forms of technical measurement (length, weight etc). Magnitude estimation doesn't require lengthy preparation, and training, of a number of standards as category scales often do and additionally removes the problem of scale end avoidance.

Assessors were given training in use of this technique prior to rating the design samples. Training consisted of two replicate sessions assessing samples varying in concentration of each of the design factors (citric acid, lactic acid, glucose, fructose or aroma volatiles). Four concentrations of each design factor were examined within the range to be included in the model beverage. For each design factor, the mid-range concentration was used as a reference sample and designated a value of 100 for sourness (citric acid and lactic acid sample set), sweetness (glucose or fructose sample set) or citrus-like flavour (aroma volatiles sample set) respectively.

Panellists were instructed to rate each set of four samples in ratio relation to the reference sample. If they perceived a sample to be twice as intense as

the reference, then it should be given a score of 200, if 1/5th as intense then a score of 20 should be assigned.

3.2.5. Sensory evaluation

All testing was performed in an air-conditioned room (18°C), under Northern Hemisphere daylight and in individual booths. Data were collected using the computerised data acquisition system, Fizz (Biosystemes, France).

For evaluation of the model design spaces, panellists were given the reference sample, containing aroma volatiles, acid and sugar (section 3.2.3); which was assigned a value of 100 for 'citrus-like' flavour intensity. This sample was from within the design space of Model 3, but was not included as a design point in assessment of this model. Aroma and tastant levels of the reference sample were chosen specifically to create a medium flavour intensity and excluded the extremes of acid and sugar ranges.

Panellists were instructed to score each test sample in terms of citrus flavour intensity in ratio relation to the reference. Panellists were instructed to taste the reference sample at the beginning of each set of three samples and it was freely available throughout the test sessions to allow panellists to re-familiarise themselves with it as needed. The same composition for the reference sample was used for all studies to allow direct comparison between sample sets.

Panellists were instructed to concentrate only on the specific attribute to be assessed and were told that they would be able to express changes in other attributes by evaluating them in later sessions. Encouraging panellists to use an analytical strategy in assessment of the samples and additionally directing attention to appropriate attributes, whilst providing prior knowledge of which attributes will be rated, has previously been suggested to remove enhancement effects due to 'dumping' errors or taste-odour confusion (Prescott, 1999, Prescott *et al*, 2004). Furthermore, rating three attributes using magnitude estimation would not have allowed the evaluation of the

complete design set in one session, introducing possible session-product interactions.

Acidity and sweetness attributes were evaluated using the same technique (but now the same reference sample was assigned a value of 100 for its acidity or sweetness intensity respectively). Collecting data on attributes associated with both volatile and non-volatile flavour components allowed the assessment of intra-modal taste interactions. Due to time and cost constraints, sourness and sweetness attributes were only evaluated for Models 3 (glucose/citric acid) and 4 (fructose/citric acid).

3.2.6. Data analysis and panel performance monitoring

Replicate scores were examined for each panellist to identify inconsistencies in sample scoring, this included analysis of scores assigned to the internal reference sample within each session. Panel performance was monitored using Fizz sensory software (Biosystemes, France). One-way analysis of variance (ANOVA) was calculated for each panellist, and individual coefficient of variance (CV) and discrimination probability values (FPROD) derived to enable individual panellist's precision (repeatability of replicate data), accuracy (proximity of scores for internal reference samples to 100) and ability to discrimination between samples (p value).

ANOVA (analysis by attribute with product and judge factors), and where appropriate, Tukey's HSD multiple comparison tests were used to determine significant differences between samples within each model design for each assessed attribute.

3.2.6.1. Generation of Predictive Models

Predictive polynomial models were generated to explain variations of perceived citrus flavour intensity as a function of sugar, acid and aroma concentration (Design Expert software, Stat-Ease Inc, Minneapolis). Non-significant terms, as determined by ANOVA, were removed and, after

scrutiny of best-fit equations and associated model values, a final mathematical model was chosen that best represented the data.

Additional design points (3) and replicate samples (3) were included within each model design to allow estimation of Lack of Fit. The Lack of Fit (LoF) test compares the residual error to the pure error from the replicated design points. A residual error significantly larger than the pure error indicates that some data variation remains in the residuals that may be removed by a more appropriate model. A significant LoF would indicate that the generated predictive model does not fit the data well and may require more terms to be included in the final equation.

The ability of the final model to explain the data was indicated by adjusted R^2 and predictive R^2 values. The adjusted R^2 value is a measure of the amount of variation about the mean explained by the model, and is obtained from the R^2 value following adjustment for the number of parameters in the model relative to the number of points in the design (Design Expert 6.0 WinHelp 2000). The predicted R^2 value is a measure of how well the model predicts a response value.

The predictive ability of these models was practically assessed by means of the evaluation of a separate validation set of samples taken from within the design space but not part of the original model data.

3.3. Results

Prior to a full data analysis and interpretation of the model results, it is essential to understand the effectiveness and reliability of the data produced. This is achieved by assessment of panel performance through the use of ANOVA derived variation coefficients, measures of panel accuracy and precision, scoring of an internal reference sample and the use of two-way ANOVA to identify judge and judge-product interactions.

3.3.1. Assessment of panel performance

Investigation of individual panellists' results enabled assessment of repeatability and discrimination. Panellist ability to score samples consistently (repeatability) was assessed by %CV values and the probability value, FPROD, indicated level of discrimination between products (Fizz software, Biosystemes). Figure 3-2 illustrates the relationship between repeatability and discriminative ability for individual panellists, for each of the 4 models. Each circle on the graphs represents data for each panellist, colour-coded to aid interpretation (see colour key at bottom of graphs). Plotting probability values and %CV together enables simple visualisation of panellists' data, points falling in the lower, right-hand portion of the plots show both good discrimination (low probability values) and repeatability (low %CV values). Figure 3-3 illustrates the mean score of the internal control reference sample for each panellist.

Visual inspection of the panel monitoring graphs (Figure 3-2) showed the majority of panellists were able to score samples effectively within the model designs, as measured by %CV, for flavour intensity. The repeatability (%CV) was below 25% for panellists 1, 2, 5, 7, 8, 10, 11 and 12 in most of the models. This was coupled with FPROD probability values below 0.1 indicating panellists were able to discriminate effectively between samples in terms of flavour intensity at a confidence level of 10% (Figure 3-2).

Panellists 3 and 4, however, showed inconsistencies in scoring, resulting in repeatability indexes above 50% in 3 out of 4 models. Panellists 3, 6 and 9 were unable to effectively discriminate between samples in 2 out of the 4 models ($P>0.1$), and panellist 4 showed poor discrimination in 3 out of 4 models ($p>0.05$, Figure 3-2).

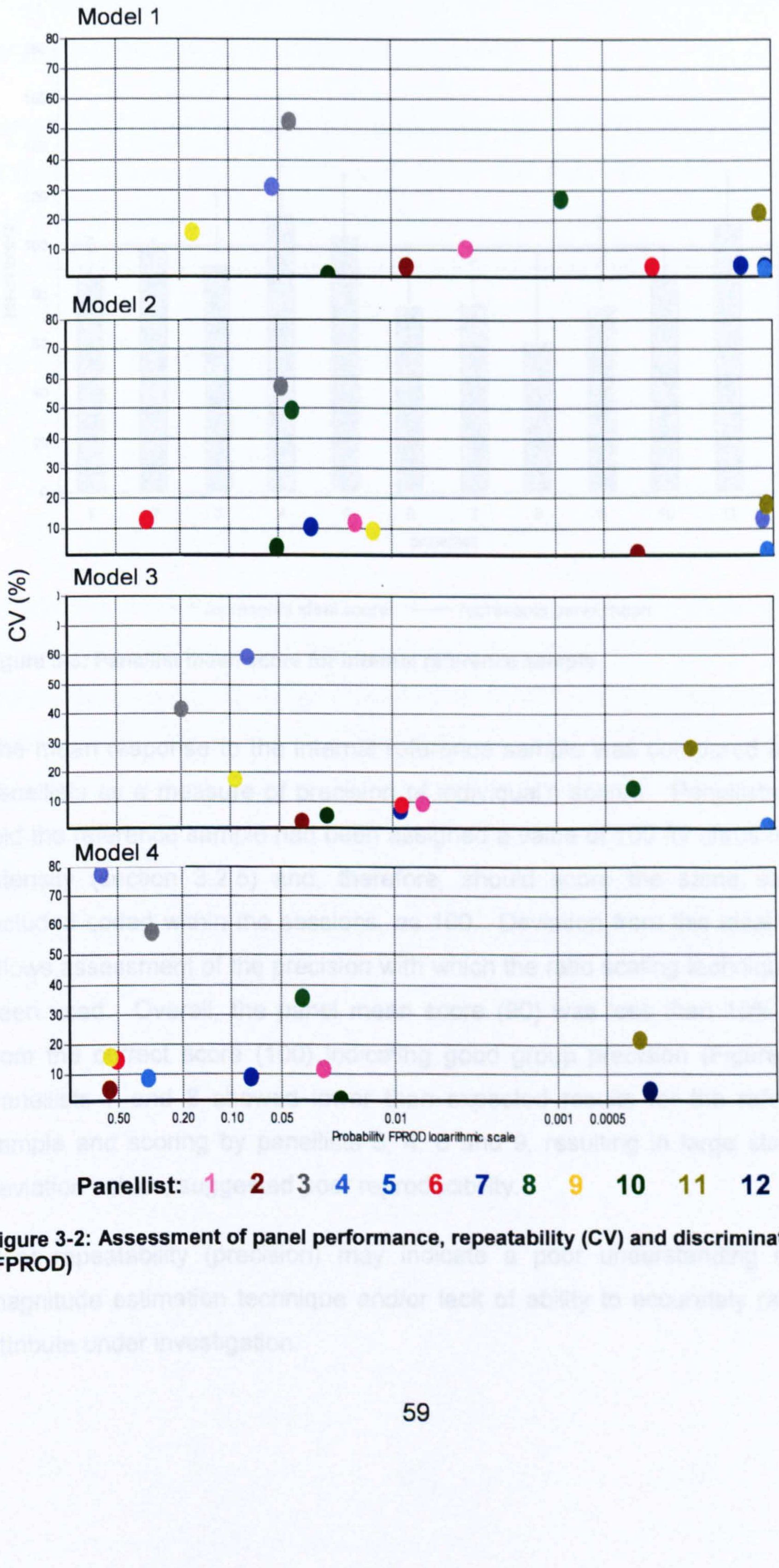


Figure 3-2: Assessment of panel performance, repeatability (CV) and discrimination (FPROD)

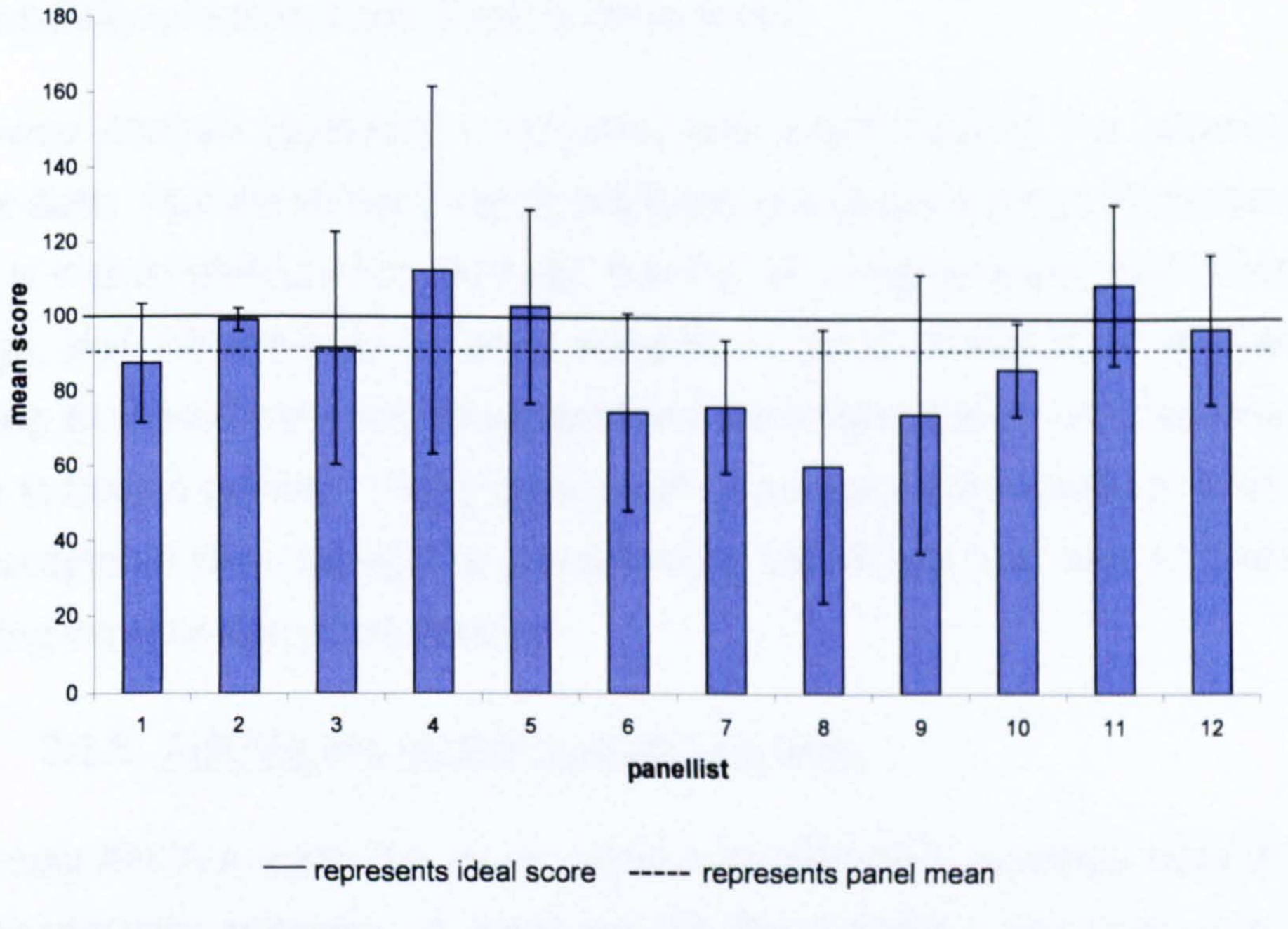


Figure 3-3: Panellist mean score for internal reference sample

The mean response to the internal reference sample was compared across panellists as a measure of precision of individual's scores. Panellists were told the reference sample had been assigned a value of 100 for citrus flavour intensity (section 3.2.5) and, therefore, should score the same sample, included coded within the sessions, as 100. Deviation from this ideal score allows assessment of the precision with which the ratio scaling technique has been used. Overall, the panel mean score (90) was less than 10% away from the correct score (100) indicating good group precision (Figure 3-3). Panellists 7 and 8 showed lower than expected results for the reference sample and scoring by panellists 3, 4, 8 and 9, resulting in large standard deviation values, suggested poor reproducibility.

Poor repeatability (precision) may indicate a poor understanding of the magnitude estimation technique and/or lack of ability to accurately rate the attribute under investigation.

Therefore, after consideration of all panel monitoring data, panellist 3 and 4 were removed from further analysis on the basis of comparatively low repeatability (precision) and discriminative ability.

Two-way ANOVA (panellist x samples) was performed on the remaining panel data. Results showed significant judge and judge-product interactions. This is mainly attributed to the large number of samples within each Model design, and the similarity of many samples in terms of assessed attributes leading to cross-over interactions between panellists, and is not uncommon even in trained panels. These cross-over interactions were deemed to be at an acceptable level, taking into consideration the sample set, and no further training was considered necessary.

3.3.2. ANOVA and multiple comparison tests

Two-way ANOVA using the mean panel data indicated panellists were able to discriminate between at least two of the samples assessed, for all attributes examined ($p>0.001$). Full ANOVA tables are included in Appendix 1. Subsequent Tukey's HSD multiple comparison tests (5% confidence level) were used to determine which samples were significantly different to each other within each model design for each attribute assessed. This multiple comparison test assigns a letter(s) to each sample; samples with the same letter have not been calculated to be significantly different from each other in terms of the rated attribute examined.

Table 3-2: Sample composition, mean panel data and post hoc test groupings for flavour intensity of Model 1.

	MODEL 1					
Sample	Sample Composition			Flavour		
	glucose (g/l)	lactic acid (ml/l)	Flavour Level	Panel mean	*(stdev)*	
1	150	1.31	2	118.00	*33.8*	ABCD
2	0	2.63	1	125.75	*79.0*	ABC
3	75	0	1	61.55	*37.9*	EF
4	150	2.63	2	149.95	*57.9*	A
5	75	0	2	83.65	*37.8*	DE
6	0	0	1	27.60	*23.4*	F
7	0	2.63	2	141.25	*56.0*	A
8	75	1.31	1	90.20	*28.6*	CDE
9	150	2.63	1	134.40	*56.5*	A
10	37.5	1.31	2	115.70	*23.5*	ABCD
11	0	0	2	62.95	*44.4*	EF
12	150	0	2	94.75	*55.9*	BCDE
13	75	0	1	65.50	*22.2*	E
14	0	2.63	1	128.75	*94.4*	AB
15	150	2.63	1	143.90	*61.4*	A
16	0	2.63	2	137.30	*68.2*	A

Samples with the same letter are not significantly different from each other ($p<0.05$)

Table 3-3: Sample composition, mean panel data and post hoc test groupings for flavour intensity of Model 2.

Sample	MODEL 2				
	Sample Composition			Flavour	
	fructose (g/l)	lactic acid (ml/l)	flavour level	Panel mean *(stdev)*	
1	64	0	1	69.44 *33.4*	BCDE
2	0	2.63	1	107.22 *90.4*	ABC
3	32	1.13	1	102.11 *20.8*	ABC
4	64	2.63	1	141.33 *63.8*	A
5	64	0	2	101.67 *38.5*	ABC
6	0	1.31	2	105.44 *30.9*	ABC
7	0	0	2	56.28 *35.7*	DE
8	32	2.63	2	138.39 *50.0*	A
9	0	0	1	35.39 *24.8*	E
10	32	0.66	2	90.28 *21.7*	BCD
11	0	2.63	2	142.39 *78.0*	A
12	64	2.63	2	140.94 *62.2*	A
13	32	0	1	64.06 *24.6*	CDE
14	0	2.63	1	111.39 *98.7*	AB
15	64	2.63	1	141.28 *76.1*	A
16	64	0	2	99.11 *42.5*	ABCD

Samples with the same letter are not significantly different from each other ($p<0.05$)

Table 3-4: Sample composition, mean panel data and post hoc test groupings for flavour, sweetness and sourness intensity of Model 3.

	MODEL 3											
Sample	Sample Composition			Flavour			Sweetness			Sourness		
	fructose (g/l)	citric acid (g/l)	flavour level	Panel mean *(stdev)*								
1	0	0	1	40.17	*29.9*	I	27.33	*24.2*	E	29.83	*32.2*	F
2	64	0	2	112.5	*51.8*	CDEFG	229.17	*98.4*	A	54.17	*25.6*	DEF
3	0	0	2	54.78	*37.6*	HI	43.56	*35.1*	E	36.50	*36.1*	EF
4	0	1.5	2	126.11	*36.1*	CDE	48.78	*25.7*	E	185.00	*66.1*	A
5	64	0.75	1	133.33	*49.3*	CD	151.22	*37.0*	BC	88.33	*21.2*	CD
6	0	1.5	1	102.78	*32.9*	DEFG	53.72	*27.9*	E	177.78	*63.8*	A
7	32	0.75	2	123.33	*42.3*	CDEF	220.61	*89.4*	A	78.33	*32.3*	CDE
8	32	0	1	74.89	*17.6*	GHI	162.94	*43.5*	B	42.50	*25.1*	EF
9	64	1.5	2	180.56	*72.4*	AB	205.00	*88.2*	A	120.56	*55.9*	BC
10	16	0.75	1	87.11	*12.6*	EFGH	119.94	*30.5*	CD	87.22	*9.5*	CD
11	64	0	1	79.39	*43.0*	GHI	227.78	*101.6*	A	40.78	*18.0*	EF
12	64	1.5	1	106.89	*32.5*	CDEFG	205.67	*76.9*	A	113.50	*50.3*	C
13	32	0	2	99.39	*36.4*	DEFG	167.67	*55.0*	B	38.33	*20.1*	EF
14	64	1.5	2	187.5	*82.2*	A	218.33	*104.1*	A	159.72	*128.1*	AB
15	0	1.5	2	145.06	*43.7*	BC	49.89	*30.3*	E	197.22	*100.8*	A
16	0	1.5	1	105.67	*67.6*	CDEFG	45.17	*28.3*	E	186.50	*76.4*	A

Samples with the same letter, within a column, are not significantly different from each other ($p<0.05$)

Table 3-5: Sample composition, mean panel data and post hoc test groupings for flavour, sweetness and sourness intensity of Model 4.

Sample	MODEL 4											
	Sample Composition			Flavour			Sweetness			Sourness		
	glucose g/l	citric acid (g/l)	flavour level	Panel mean *(stdev)*								
1	0	1.5	1	118.75	*46.2*	ABCDE	32.11	*29.6*	J	184.95	*88.2*	A
2	150	0	1	66.65	*33.1*	GH	242.33	*95.8*	A	36.75	*27.6*	E
3	0	0	1	48.45	*32.9*	H	40.89	*30.0*	J	30.60	*34.5*	E
4	150	0.75	2	121.00	*36.6*	ABCDE	216.28	*83.6*	ABC	85.50	*54.4*	CD
5	75	0.75	1	104.00	*18.8*	BCDEFG	152.06	*45.0*	DEF	86.75	*22.4*	CD
6	0	1.5	2	135.25	*55.2*	ABC	53.33	*28.0*	HIJ	187.15	*83.0*	A
7	75	0	2	100.00	*24.8*	CDEFG	140.78	*31.8*	EF	44.25	*19.0*	E
8	0	1.5	2	151.00	*61.6*	A	180.11	*74.0*	CDE	129.00	*85.3*	B
9	150	1.5	1	129.35	*55.2*	ABCD	210.28	*90.7*	ABC	119.55	*42.7*	BC
10	37.5	0.75	2	110.25	*26.4*	ABCDEF	126.72	*40.1*	FG	100.05	*12.6*	BC
11	75	0	1	69.70	*30.6*	FGH	48.89	*34.4*	HIJ	42.40	*41.8*	E
12	150	0	2	108.25	*29.7*	BCDEF	225.56	*83.2*	AB	59.75	*29.7*	DE
13	0	0	2	83.80	*18.9*	EFGH	157.28	*35.6*	DEF	39.50	*21.4*	E
14	150	1.5	1	125.90	*38.9*	ABCD	184.78	*49.7*	BCD	121.35	*54.8*	BC
15	0	1.5	1	124.95	*46.5*	ABCD	45.89	*32.8*	IJ	197.05	*89.8*	A
16	150	1.5	2	143.05	*58.3*	AB	47.44	*23.9*	HIJ	186.00	*82.0*	A

Samples with the same letter, within a column, are not significantly different from each other ($p<0.05$)

Table 3-4 and Table 3-5 describe the sample composition, mean panel data (and standard deviation) and the multiple comparison test groupings for each of the four model designs and for each attribute evaluated (flavour intensity for models 1 and 2 and flavour, sweetness and sourness intensities for models 3 and 4). This data is provided for the reader reference hereinafter and are further discussed in terms of predicted models generated from these results.

3.3.3. Predictive models

Using the panel mean values, significant predictive polynomial models were generated using Design Expert, which described the perceptual results in terms of the design factors (aroma, sugar, acid) used in each experiment.

Significant terms, as determined by ANOVA ($p<0.05$), were included in the final mathematical model, which was chosen to best represent the data after scrutiny of best-fit equations and associated model values. These model statistics are useful in determining how well the predictive models fit the experimental data and are calculated by Design Expert software (Design Expert 6.0, Stat-Ease Inc, Minneapolis) and defined as described in the following section (Design Expert 6.0, WinHelp 2000).

The PRESS statistic (Predicted Residual Error Sum of Squares) is a measure of how the model fits each point in a design. It is calculated by first predicting where each point should be from a model that contains all other points except the one in question. The squared residuals (difference between actual and predicted values) are then summed. R-squared (R^2) values indicate the amount of variation around the mean explained by the model and are calculated by Equation 2.

$$R^2 = 1 - (SS_{residual}) / (SS_{model} + SS_{residual})$$

where $SS_{residual}$ is the sum of squares of residual variation
SS_{model} is the sum of squares of model variation

Equation 2: Calculation of R-squared values

Adjusted R-squared (adj R^2) values are this measure adjusted for the number of terms in the model and are calculated by Equation 2.

$$Adj\ R^2 = 1 - ((SS_{residual}/DF_{residual}) / ((SS_{model} + SS_{residual})/DF_{model} + DF_{residual})))$$

Where: $SS_{residual}$ is the sum of squares of residual variation,
$DF_{residual}$ is the degrees of freedom associated with the residual variation,
SS_{model} is the sum of squares of model variation
DF_{model} is the degrees of freedom associated with the model variation

Equation 3: Calculation of adjusted R-squared values

Predicted R-squared (pred R^2) values are a measure of the amount of variation in new data explained by the model (Equation 3).

$$pred\ R^2 = 1 - (PRESS/SS_{total})$$

Where: SS_{total} is the sum of squares of the total variation
PRESS is the predicted residual error sum of squares

Equation 4: Calculation of predicted R-squared values

Adequate precision is a signal to noise ratio. It compares the range of predicted values at the design points to the average prediction error. Ratios greater than 4 indicate adequate model discrimination.

Predictive models were generated for each model design and each attribute assessed. Lack of Fit was not significant for any model indicating the residual error was not significantly larger than the pure error associated with the models.

The design factors involved in each predictive equation, together with the model statistics described above, are shown below (Table 3-6).

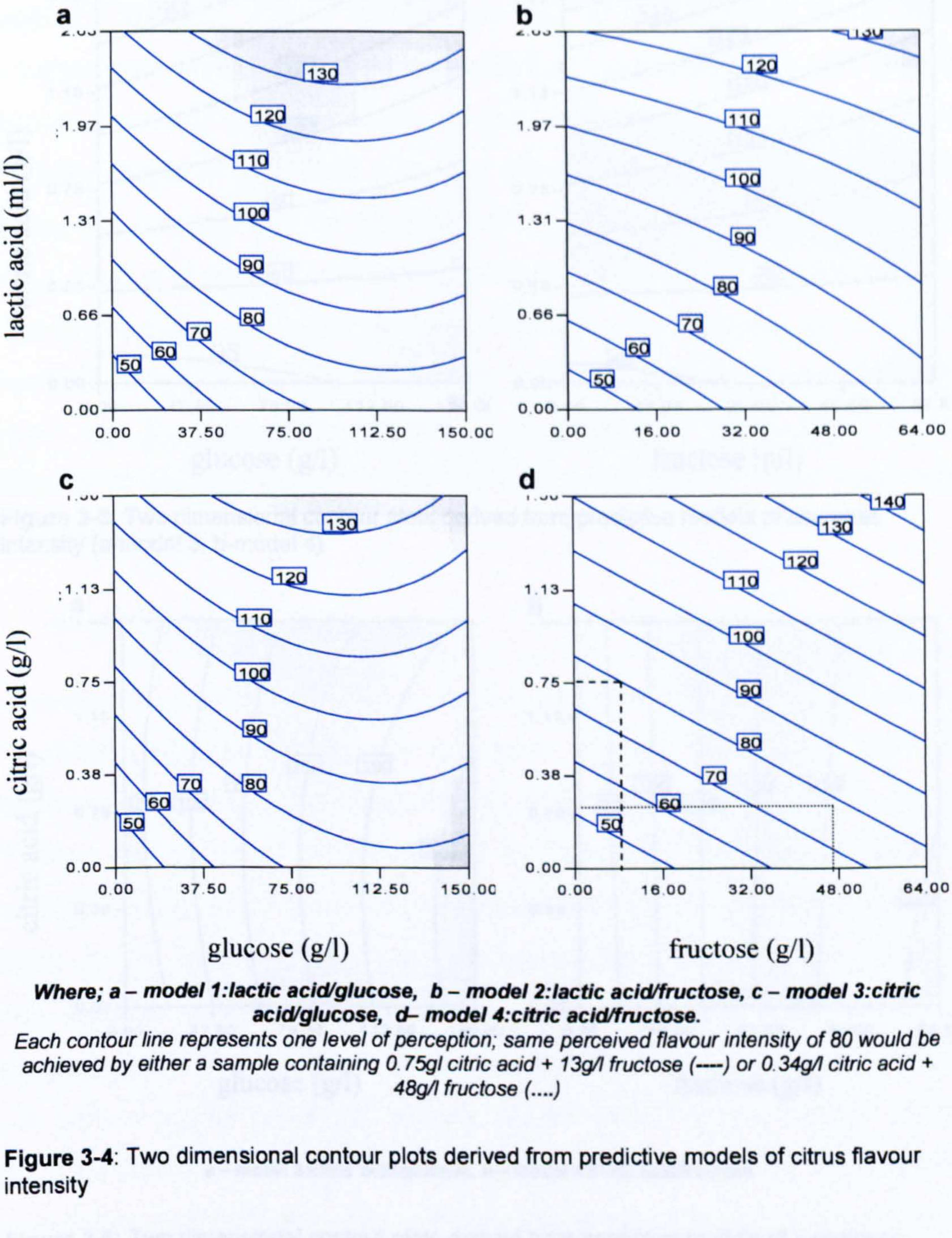
Table 3-6 Predictive intensity models in terms of significant design composition factors for each model design and each attribute examined.

				significant model terms					model statistics				
attribute		aroma volatile level	intercept	sugar	acid	sugar^2	acid^2	sugar*acid	PRESS	R^2	Adj R^2	Pred R^2	Adeq Precision
citrus-like flavour intensity	**model 1** glucose/lactic acid	1	38.5	+ 0.57	+ 30.1	- 0.002		- 0.005	2979.22	0.95	0.94	0.92	30.13
		2	61.7	+ 0.57	+ 30.1	- 0.002		- 0.005					
	model 2 fructose/lactic acid	1	42.3	+ 0.62	+ 29.4			- 0.15	3029.25	0.95	0.94	0.92	30.34
		2	61.3	+ 0.62	+ 29.4			- 0.15					
	model 3 glucose/citric acid	1	38.2	+ 0.63	+ 51.4	- 0.003		- 0.11	2239.67	0.96	0.95	0.94	38.96
		2	60.5	+ 0.63	+ 51.4	- 0.003		- 0.11					
	model 4 fructose/citric acid	1	39.6	+ 0.57	+ 49.9				5528.29	0.92	0.91	0.88	30.47
		2	67.9	+ 0.57	+ 49.9								
sweetness intensity	**model 3** glucose/citric acid	1	39.0	+ 1.73	+ 43.0	- 0.003	- 25.5	- 0.20	3147.57	0.99	0.98	0.97	38.36
		2	39.0	+ 1.73	+ 43.0	- 0.003	- 25.5	- 0.20					
	model 4 fructose/citric acid	1	38.4	+ 4.90	+ 7.50	- 0.003		- 0.30	979.28	0.99	0.99	0.99	56.79
		2	38.4	+ 4.90	+ 7.50	- 0.003		- 0.30					
sourness intensity	**model 3** glucose/citric acid	1	30.3	+ 0.10	+ 76.7		+ 17.0	- 0.30	3620.59	0.98	0.98	0.96	31.49
		2	38.4	+ 0.10	+ 76.7		+ 17.0	- 0.30					
	model 4 fructose/citric acid	1	34.4	+ 0.20	+ 64.9		+ 24.8	- 0.72	955.15	0.99	0.99	0.99	65.67
		2	34.4	+ 0.20	+ 64.9		+ 24.8	- 0.72					

Contour plots illustrating the predicted sensory intensity from the model data are shown in Figure 3-4 (flavour attribute), Figure 3-5a-b (sourness attribute) and Figure 3-6a-b (sweetness attribute). Both volatile levels 1 (2.5ppm citral and limonene) and 2 (10ppm citral and limonene) resulted in the same pattern of variation in perception for each attribute (and have the same predictive equation aside from an increased weighting for volatile level 2), therefore, only contour plots dissecting the 3D predictive models at volatile level 1 are shown.

The contour plots provide a visual representation of the models. Each contour line represents one sensory intensity score, with varying concentrations of acid and sugar shown along the plot axis. For example, in Figure 3-4d, the same perceived flavour intensity of 80 would be achieved by either a sample containing 0.75gl citric acid + 8g/l fructose (----) or 0.25g/l citric acid + 48g/l fructose (....). In this way, the effect on flavour perception of varying acid and sugar concentrations can be easily seen across the design spaces.

Analysis of the contour plots showed that lactic and citric acid increased the intensity of perceived citrus flavour, and that this enhancement is consistent across the design space (Figure 3-4). Both fructose and glucose enhanced perception of flavour but, whilst this effect was uniform across the design space for fructose (Figure 3-4b and d), glucose showed a flattening out of enhancement at concentrations above 110g glucose/L (Figure 3-4a and c). These differences are reflected in the predictive equations generated (Table 3-6); the equations for models 2 and 4 (combinations of acids and fructose) are linear whilst those for models 1 and 3 (combinations of acids and glucose) are quadratic, indicating a more complex relationship for glucose's impact on perception and visually describing the plateau effect of mid-high levels of glucose on flavour perception.



Where; a – model 1:lactic acid/glucose, b – model 2:lactic acid/fructose, c – model 3:citric acid/glucose, d– model 4:citric acid/fructose.

Each contour line represents one level of perception; same perceived flavour intensity of 80 would be achieved by either a sample containing 0.75gl citric acid + 13g/l fructose (----) or 0.34g/l citric acid + 48g/l fructose (....)

Figure 3-4: Two dimensional contour plots derived from predictive models of citrus flavour intensity

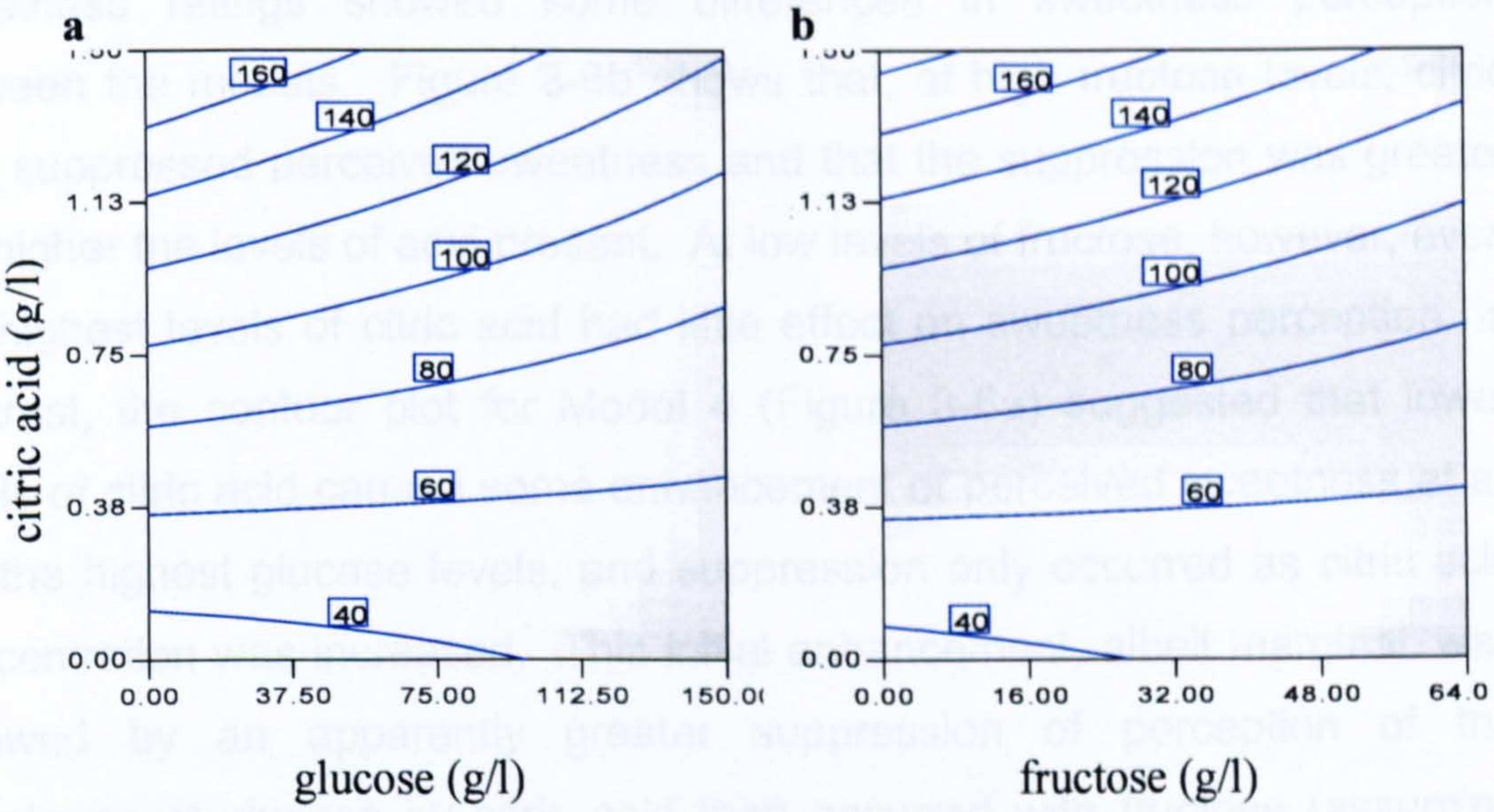
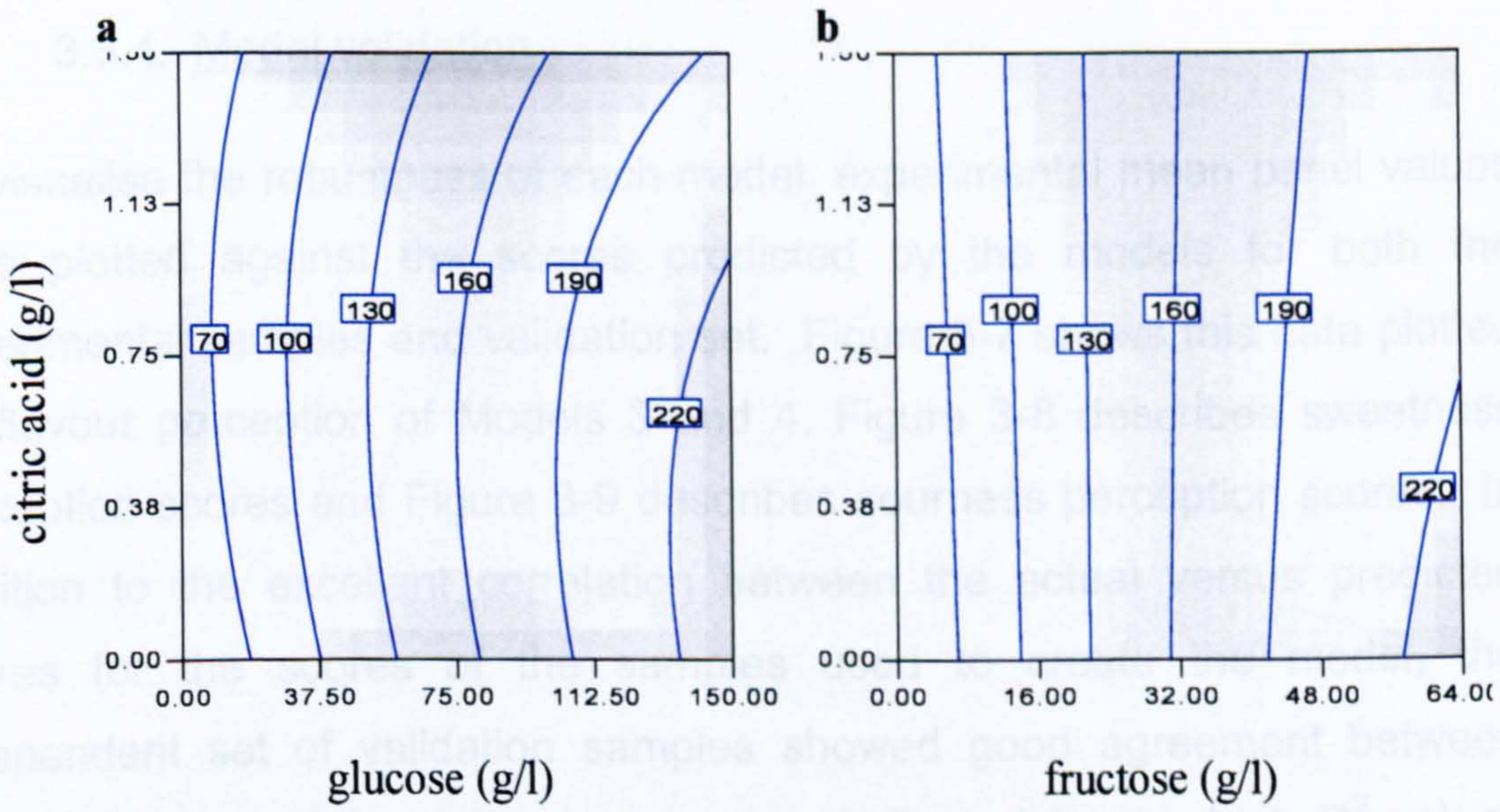


Figure 3-5: Two dimensional contour plots derived from predictive models of sourness intensity (a-model 3, b-model 4)



a – model 3:citric acid/glucose, b – model 4:citric acid/fructose

Figure 3-6: Two dimensional contour plots derived from predictive models of sweetness intensity (a-model 3, b-model 4).

The contour plots and predictive equations resulting from analysis of sourness attribute ratings showed that the addition of both sugars results in

suppression of sourness perception (Figure 3-5, Table 3-6). The analysis of sweetness ratings showed some differences in sweetness perception between the models. Figure 3-6b shows that, at high fructose levels, citric acid suppressed perceived sweetness and that the suppression was greater the higher the levels of acid present. At low levels of fructose, however, even the highest levels of citric acid had little effect on sweetness perception. In contrast, the contour plot for Model 4 (Figure 3-6a) suggested that lower levels of citric acid caused some enhancement of perceived sweetness at all but the highest glucose levels, and suppression only occurred as citric acid concentration was increased. This initial enhancement, albeit marginal, was followed by an apparently greater suppression of perception of the sweetness of glucose by citric acid than occurred with fructose (assuming equi-sweet levels).

3.3.4. Model validation

To visualise the robustness of each model, experimental mean panel values were plotted against the scores predicted by the models for both the experimental samples and validation set. Figure 3-7 shows this data plotted for flavour perception of Models 3 and 4, Figure 3-8 describes sweetness perception scores and Figure 3-9 describes sourness perception scores. In addition to the excellent correlation between the actual versus predicted scores for the scores of the samples used to create the model, the independent set of validation samples showed good agreement between actual versus model predicted scores, as indicated by the high R^2 values (refer to section 3.2.6.1). As this sample set was not involved in generating the predictive model, these results indicate that the models could be used to accurately predict/describe new data from within the design space.

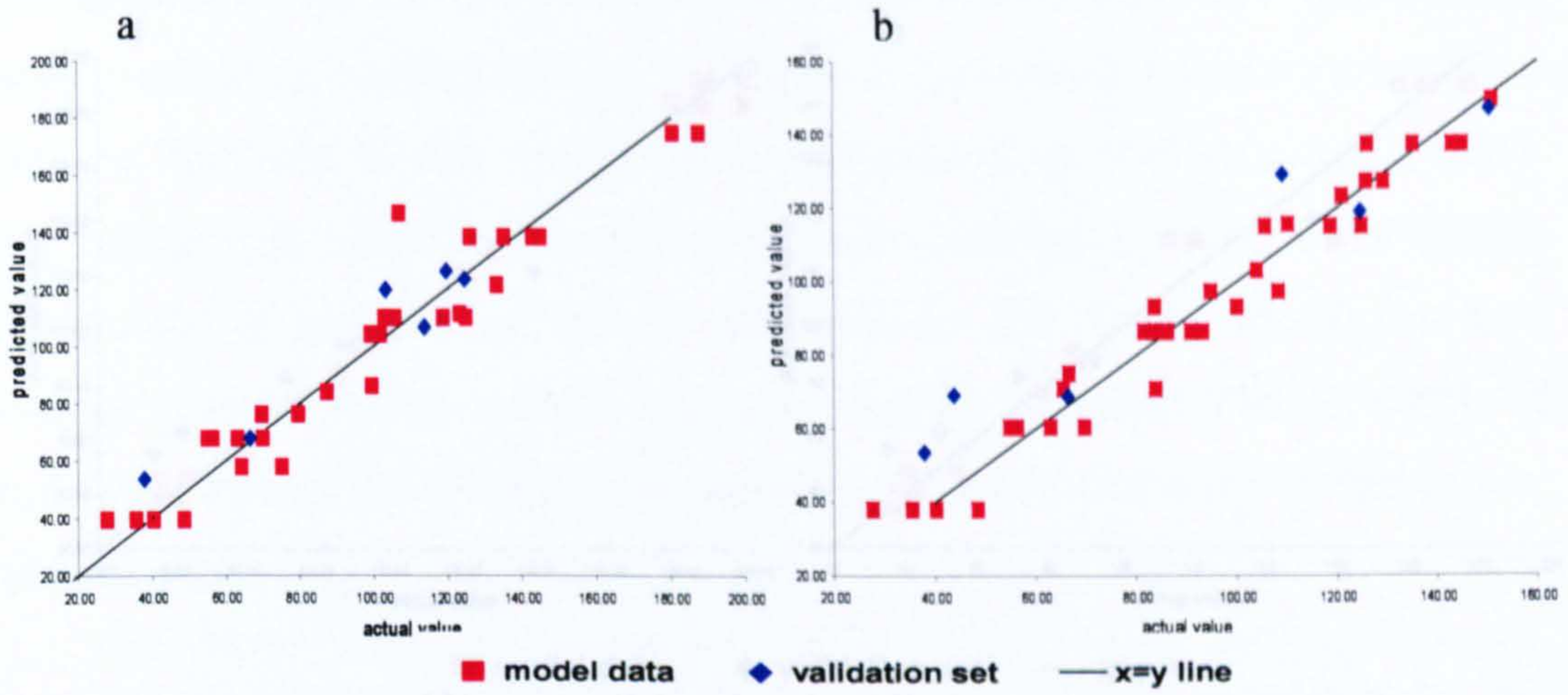


Figure 3-7: Predicted versus actual scores for flavour intensity of both model samples and validation set (a-model 3, R^2= 0.92, b-model 4, R^2= 0.93).

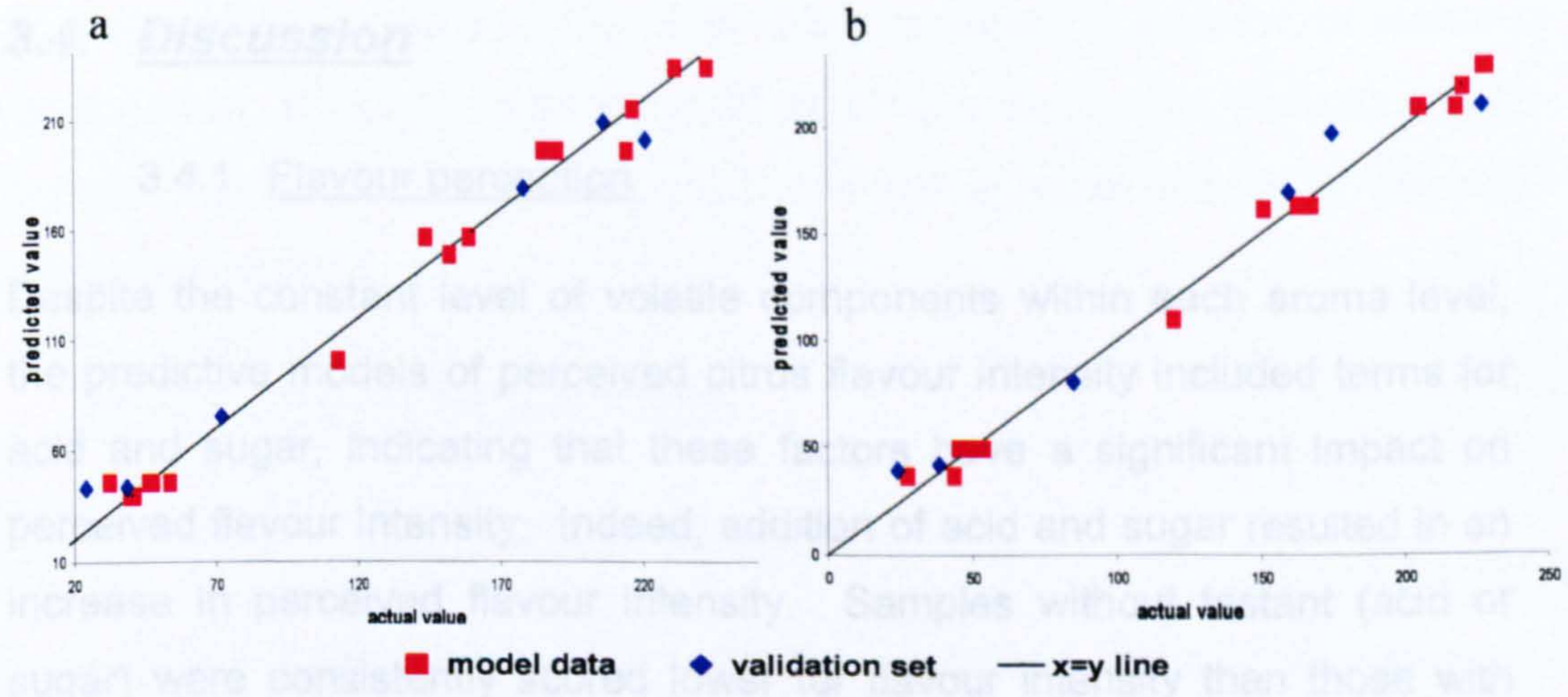


Figure 3-8: Predicted versus actual scores for sweetness intensity of both model samples and validation set (a-model 3, R^2= 0.97, b-model 4, R^2= 0.99).

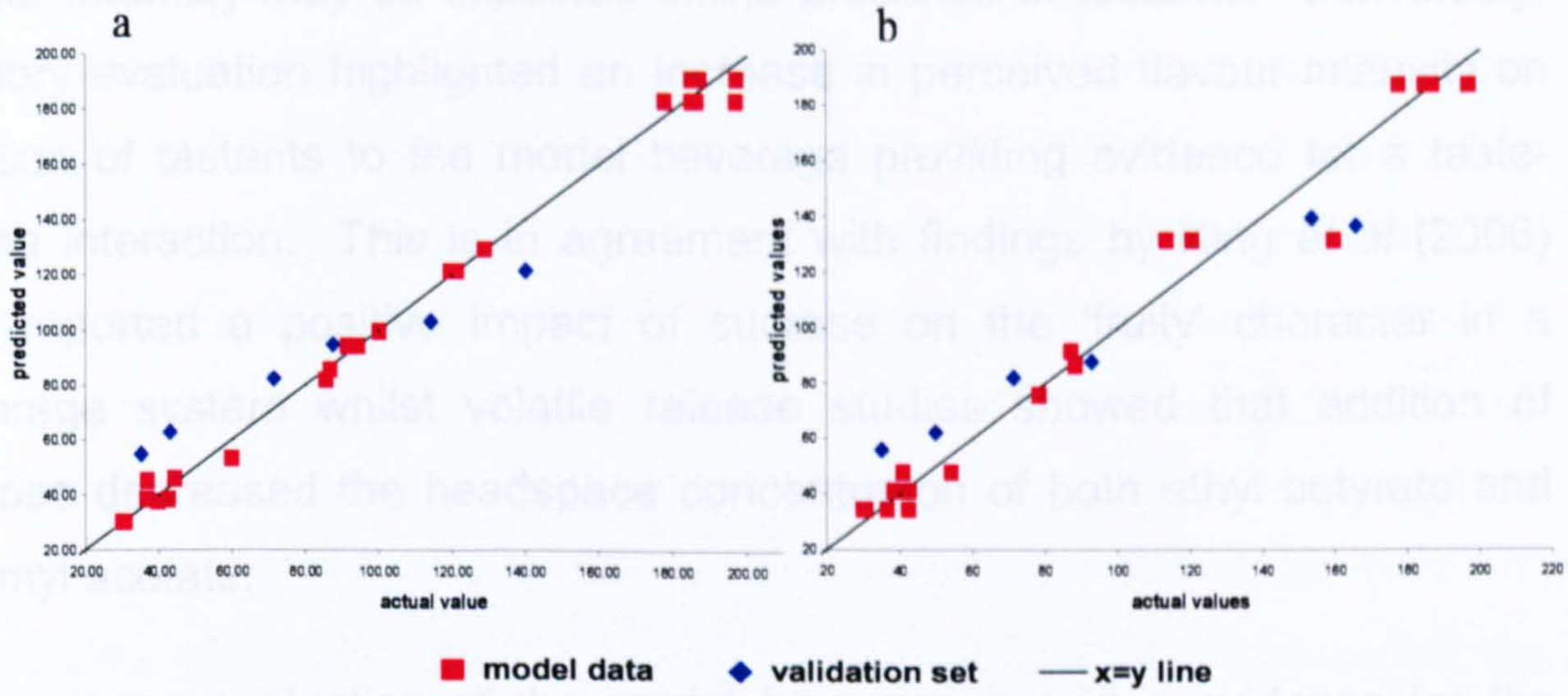


Figure 3-9: Predicted versus actual scores for sourness intensity of both model samples and validation set (a-model 3, R^2= 0.98, b-model 4, R^2= 0.98).

3.4. Discussion

3.4.1. Flavour perception

Despite the constant level of volatile components within each aroma level, the predictive models of perceived citrus flavour intensity included terms for acid and sugar, indicating that these factors have a significant impact on perceived flavour intensity. Indeed, addition of acid and sugar resulted in an increase in perceived flavour intensity. Samples without tastant (acid or sugar) were consistently scored lower for flavour intensity than those with tastant (Table 3-2, 3-3, 3-4 and 3-5). This effect was seen with all tastants examined, i.e. addition of glucose, fructose, citric acid or lactic acid, either alone or in combination, caused an increase in the sensory perception of the citrus-flavour intensity.

Previous analysis of headspace concentration of volatiles (Chapter 2, section 2.3.1) revealed that addition of tastants resulted in a modification of volatile release. Compared to the aroma volatiles-water only sample, the presence of tastants within the aqueous phase decreased the amount of volatiles

measured in the headspace at equilibrium. This suggested a reduction in flavour intensity may be expected in the presence of tastants. Conversely, sensory evaluation highlighted an increase in perceived flavour intensity on addition of tastants to the model beverage providing evidence for a taste-aroma interaction. This is in agreement with findings by King *et al* (2006) who reported a positive impact of sucrose on the 'fruity' character in a beverage system whilst volatile release studies showed that addition of sucrose decreased the headspace concentration of both ethyl butyrate and isoamyl acetate.

The sensory evaluation of the model beverage provides evidence for the presence of taste-aroma interactions within this system. Addition of the sugars and acids increased the ratings of citrus flavour intensity compared to the absence of tastants. This does not appear to be directly related to modification of the release profile of citral or limonene on addition of tastants. Taken together, these findings suggest that the presence of tastants results in changes in flavour perception which are not a consequence of physico-chemical interactions.

3.4.2. Cross-modal interactions

3.4.2.1. Acid-aroma interactions

Analysis of the contour plots describing the perceived citrus flavour intensity data show steeper gradients for contour lines relating to increasing acid levels than sugar levels. Thus, acidity appears to be a key driver in determining perceived flavour intensity. Both citric and lactic acid, in the absence of sugar, result in similar levels of perceived flavour intensity which, as these are equi-sour levels, suggests that both these organic acids enhance flavour perception to a similar magnitude.

Several previous studies have shown congruency of flavour-tastant pairings to be important in predicting perception (Frank *et al.* 1988; Schifferstein 1995). It is interesting to note that in the present studies, both acids showed

very similar results. The expectation may have been that citric acid would have a greater congruency in this citrus flavoured system than lactic acid, which is more commonly associated with milk/yoghurt systems. Previous work by Pfeiffer *et al* (2006) found differences in enhancement of a strawberry flavour both between organic and inorganic acids and within the organic acid group. Citric and malic acids both showed a greater enhancement of perceived flavour intensity than lactic acid, and an inorganic acid (phosphoric acid) which showed initial enhancement followed by suppression at higher concentrations. The authors concluded that citric and malic acids, which are naturally found in many fruits, may produce a greater effect on flavour enhancement than lactic or phosphoric acid due to a greater congruency between the taste-aroma pairing.

Pfeiffer's study, however, used equi-ratio amounts of each acid and did not take into account variations in perceptual sourness elicited. In the present study, differences in sourness perception have been reduced by using equi-sour ranges of citric and lactic acid (literature based values, (Savant *et al.* 2004)), and this may account for the similar effects on flavour perception attained for citric and lactic acids. In addition, citrus aroma is more congruent with sourness per se and it could be postulated that acid of any taste quality could enhance flavour perception.

3.4.2.2. Sugar-aroma interactions

Both sugars examined enhanced perceived flavour intensity, but although the levels used were equi-sweet, fructose appeared to sustain this enhancement effect over a wider concentration range than glucose. In Models 2 and 4 (fructose/lactic acid, fructose/citric acid, Figure 3-4b and d), fructose enhanced flavour perception and appeared to be almost as influential as the acid component (perceived flavour intensity contour lines show near symmetry). This effect is consistent across the fructose concentration range; increasing fructose concentration caused an increase in citrus flavour perception at all levels of fructose contained within the model design spaces.

Glucose, however, appears to behave differently. As the contour plots describing the data from models 1 and 3 (glucose/lactic acid and glucose/citric acid, Figure 3-4a and c) show, the influence of glucose differs depending on its concentration. Previous work by Cliff and Nobel (1990) found no effect of glucose on perceived intensity of a peach flavouring. In the present study, however, lower levels of glucose (less than 75g/L) enhanced flavour perception in a manner similar to fructose (at equi-sweet levels) resulting in similar levels of perceived flavour intensity. When present at mid-high concentrations (75-135g/L), the enhancement of perceived flavour intensity caused by the addition of glucose decreased such that further addition of glucose had little affect on the flavour intensity, as indicated by the flattening of the contour lines. Finally, the highest concentrations of glucose appeared to suppress perceived flavour intensity, the gradient of the contour lines began to increase indicating any glucose addition at these levels resulted in a decrease in the perception of intensity of citrus flavour.

Instrumental measures of viscosity (Chapter 2, section 2.3.3) found that the addition of 150g/L glucose resulted in a significant increase in viscosity compared to the addition of up to 100g/L glucose or 60g/L fructose. This suggests that samples containing the maximum concentrations of glucose or fructose, although not perceivably different in sweetness intensity, differ in viscosity. The viscosity difference is small (0.4mPa.s), and a previous study by Kappes *et al* (2006) suggested that a viscosity change of this magnitude would not result in perceivable differences in mouth. Using descriptive analysis of lemon/lime flavoured beverages, Kappes *et al* found no significant differences in 'body' or 'mouthcoating' attributes between diet and regular beverages. This result implies the addition of natural sweeteners, which are likely to increase viscosity compared to artificial sweeteners, does not lead to perceivable differences in mouthfeel attributes.

Furthermore, instrumental analysis of static headspace volatile concentrations (Chapter 2, section 2.3.1) did not provide evidence to suggest that any increase in viscosity, due to addition of 150g/L glucose, significantly modified aroma volatile release compared to samples containing other levels of tastants.

It would appear that perceptual differences in flavour intensity between equi-sweet levels of glucose and fructose are not fully explained by physico-chemical modifications. The effect of viscosity, however, on perception within this beverage system has not been fully investigated with regard to mouthfeel and so cannot be disregarded. It is conceivable that the differences in flavour intensity ratings observed between mid-high ranges of glucose and fructose may be a consequence of alterations in mouthfeel perception or taste quality directly attributable to differences in viscosity.

A review of published literature investigating sweet receptor, leads to an alternative hypothesis, based on receptor interactions, for the differences observed on flavour perception between the two sugars.

It has been previously postulated that glucose may have a separate receptor site or mechanism to fructose and other sugars (Eylam *et al.* 1998; Savant *et al.* 2004). Insect studies have proposed the existence of two receptor sites, a pyranose or P-site where glucose binds and a furanose or F-site for fructose on the sugar receptor cell (Schmidt *et al.* 1991). This may also apply to humans and indeed, studies by Eylam *et al* (Eylam *et al.* 1995; Kennedy *et al.* 1997; Eylam *et al.* 1998) have provided some evidence for this by identifying cases of hypogeusia for either fructose or glucose in the human population.

As previously mentioned (3.1.1), the human sweet receptor is thought to be a G protein-coupled heteromeric receptor made up of subunits. Recently, Nie et al (2005) have shown distinct contributions of two subunits (T1R2 and T1R3) to the detection of sweet stimuli. Interestingly, they found that sucrose

binds with a higher affinity to the T1R3 subunit than to T1R2, with the opposite being true of glucose, whilst Damak et al (2003) found that recordings of chorda tympani nerve responses of T1R3 knock-out mice were markedly reduced in response to sucrose and fructose but unaltered in response to glucose when compared to wild-type.

It is tempting to conclude that these data point to the possibility that the sweet receptor subunit with which glucose and fructose interact differ and differing binding affinities provides a plausible explanation for differences in sweetness perception at equi-molar levels.

In this study, however, the sugars were used at equi-sweet ranges, (confirmed by the sensory evaluation) and, therefore, it is difficult to reconcile the disparity between effects of glucose and fructose, at these concentrations, on flavour perception with the evidence that both provide an equal sensation of sweetness. Damak *et al* (2003) and Zhao *et al* (2003) have reported findings suggesting a functional homodimeric T1R3:T1R3 receptor. Mapping studies have identified T1R2 and T1R3 coexpression in circumvallate, foliate and palate taste cells but also a non-overlapping T1R3 distribution within a subset of fungiform and palate taste cells (Nelson *et al.* 2001). The T1R3 homodimeric receptor appears to be only activated by very high concentrations (>300mM) of natural sugars rather than artificial sweeteners (Zhao *et al.* 2003). These findings suggest T1R3 alone may function as an additional low affinity sweet receptor.

Regional differences in the expression of taste cells on the tongue result in differential responses of the nerves innervating taste cells; the chorda tympani (CT), and glossopharyngeal (GL) nerves (Ninomiya *et al.* 1997; Spector *et al.* 1997). The findings of Spector *et al* (1997) suggested that the two sugars examined (sucrose and maltose) may activate different sweet receptors (especially at high concentrations), differentially distributed in the oral cavity, and that this may be a basis for discrimination even if the sugars produce qualitatively identical sensations.

This raises the intriguing possibility that glucose and fructose may differ not only in the make-up of the taste receptors they interact with, but that, at high concentrations, their binding may consequently trigger neuronal activation that ultimately results in the differing perceptual effects observed between the two monosaccharides. Obviously, a great deal more work is required in this area but the burgeoning field of research relating to the sweet receptor may yield information on sweetener-receptor interaction consequences which could have implications for modification of perception.

3.4.2.3. Aroma-taste interactions

Interestingly, in these studies the level of volatile has little impact on sourness or sweetness perception (Figures 2a-b and 3a-b). Previous studies have suggested that taste-aroma enhancement appears to be reciprocal if pairing is congruent, 'sweet-smelling' aromas increase perceived sweetness and suppress sourness (Frank *et al.* 1989); (Djordjevic *et al.* 2004). In addition, a limited effect can be seen with imagined odours (Djordjevic *et al.* 2004). Given the congruency of the pairing of citrus-like aroma and acid, and the enhancement of citrus flavour intensity seen on addition of both citric and lactic acid in this study, it may be hypothesised that perceived sourness intensity would be enhanced by increasing citrus aroma. Model 4 (fructose/citric acid) showed no difference in sourness intensity scores due to aroma concentration (Figure 2a) whilst model 3 (glucose/citric acid) showed only a minimal enhancement effect.

There appears to be no effect of flavour level on sweetness intensity in this system (Figures 3a-b). Previous studies have reported enhancement of sweet taste in sucrose solutions containing a 'sweet' aroma (Frank *et al.* 1989; Prescott 1999). The lack of significant modification of sweet taste by the aroma compounds (citral and limonene) in the present study may be a result of congruency effects; whereby citrus flavouring is not harmonious when paired with sweetness. These findings support recent work by King *et al* (2007), who reported a lack of gustatory enhancement in sweet ratings

when maltol or vanillin were added to a sweetened apple beverage. Conversely, the same group reported a decrease in scoring of sourness of the same samples.

3.4.3. Intra-modal interactions

To examine intra-modal interactions between the tastants in this system, and to investigate further differences between the behaviour of the two sugars, the sourness and sweetness attributes were analysed and predictive models generated (Figure 3-5 and Figure 3-6).

Ratings of flavour intensity show possible evidence of mixture suppression whereby the perceived flavour intensity in response to both acid and sugar combined is less than the addition of responses to the two tastants alone (Figure 3-4). In agreement with published literature (Pangborn 1961; Schifferstein *et al.* 1990; Keast *et al.* 2003), sweetness and sourness ratings provide some evidence of mutual suppression occurring between acid and sugars (Figure 3-5 and Figure 3-6). This finding may explain why the perceived flavour intensity resulting from assessment of samples with high acid and sugar content is less than the simple addition of flavour enhancement achieved as a result of acid and sugar alone (Figure 3-4).

3.4.3.1. Sourness perception

A comparison of the contour plots describing sourness intensity (Figure 3-5) demonstrate that the addition of sugar was accompanied by a reduction of perceived sourness but suggested little difference in the level of suppression of sourness of citric acid by either fructose or glucose. This is in contrast to Savant and McDaniel (2004) who found fructose to be a more effective suppressor of citric acid sourness than glucose, however they looked at concentrations of acid up to 4.8g/l; much higher than used here, which may account for the difference. In this study, both sugars caused suppression of perceived acidity which is more marked at higher sugar levels. This agrees with published data showing less sourness suppression with increased citric

acid concentrations and more sourness suppression as the level of sugar increases.

3.4.3.2. *Sweetness perception*

The contour plots of sweetness intensity (Figure 3-6) confirm the levels chosen were equi-sweet (in absence of acid, sweetness intensity showed similar values for both sugars), and suggested the two sugars do not differ in psychophysical function – equi-sweetness is maintained across the ranges examined. There were differences in how the sugars were perceived when in solution with citric acid and this may explain the lack of influence of mid-high concentration glucose on perceived flavour intensity when presented in combination with acid. The finding that addition of acid had variable effects on sweetness of a sugar solution was not altogether surprising; previous literature has shown suppression (Pangborn 1961); little or no effect (Curtis *et al.* 1984; McBride *et al.* 1987); and enhancement (Kamen *et al.* 1961) of sweetness by acid.

Despite the finding that the highest concentration of glucose examined caused an increase in instrumentally measured viscosity (Chapter 2, section 2.3.3), the perception of sweetness appeared to increase proportionally to the amount of glucose present. Previous studies (Cook *et al.* 2005; Brossard *et al.* 2006) suggest that increasing viscosity leads to a decrease in the perceptual sweetness of sugars, so this finding would indicate sweetness perception remains unaltered by the small increase in instrumental viscosity observed with the highest glucose concentration.

3.5. Conclusions and summary

This study has successfully created a citrus-flavoured, model beverage system to investigate taste-aroma interactions and their effects on perception. Sensory evaluation, using a trained panel of assessors and the technique of magnitude estimation, allowed generation of predictive models

describing the relationship between variation in beverage components and flavour intensity. Statistical data indicate these predictive models were robust and reliable and models were subsequently validated using an independent sample set. Excellent agreement between actual ratings and predicted ratings, for both the model samples and validation set, was evident suggesting the models were capable of accurately predicting new data.

Previous investigation of physico-chemical interactions within this model beverage system suggests modification of volatile release on addition of tastants would result in reduced perception of flavour intensity (Chapter 2, section 2.3.1). Conversely, this was not supported by data from sensory evaluation of the model beverages. Addition of tastants resulted in an enhancement of perceived citrus flavour. Citric acid, lactic acid (equi-sour range) and fructose caused a concentration-dependant enhancement of flavour intensity, whilst glucose had a reduced enhancement ability at mid to high concentrations (Figure 3-4). This was despite the range of glucose and fructose being chosen to be perceptually equi-sweet, thus suggesting that this difference is not due to perceptual differences in sweetness between the two sugars and indeed no differences were observed in perception of their sweetness or their ability to suppress the sourness of citric acid (Figure 3-5 and Figure 3-6).

However, potential psychological errors, due to limiting response options within the sensory assessment, cannot be excluded from impacting on the resultant data. Previous studies provide evidence that taste-aroma interactions may result from a convergence of gustatory and olfactory stimuli (Dalton *et al.* 2000; Labbe *et al.* 2007) and reports of bi-modal neurons activated by taste and aroma stimuli (Small *et al.* 1997; de Araujo *et al.* 2003; Small *et al.* 2004; Small *et al.* 2005) suggest that this may be a result of neural integration.

The increase in viscosity seen instrumentally on addition of 150g/L glucose (Chapter 2, section 2.3.3), was not sufficient to cause alterations in volatile

release as determined by static headspace volatile concentration studies, although this may contribute to the reduced enhancement of flavour perception via mouthfeel influences.

Taken together, the data provides some evidence to suggest that the observed effects, of varying concentration and type of both of sugar and acid, seen on perceived citrus flavour may be cognitive in origin.

4. Influence of carbonation

4.1. *Introduction*

Carbonated beverages are widely consumed and extremely popular in western society. Notwithstanding decline over recent years in favour of more 'natural' non-carbonated juices and mineral waters, carbonated beverages still make up the lions share of the booming soft drink market. Recent developments have included expansion into the so-called 'energy' drink market with brands such as Red Bull becoming a seemingly overnight success and currently generating sales in excess of $1 billion worldwide (International 2007). These 'energy' or 'stimulant' drinks are typically very high in sugar and caffeine compared to the rest of the soft drink market, and often contain natural ingredients such as guarana, taurine, ginseng, ginkgo biloba and vitamins.

As the perceptual profile of a commercial beverage is its key sensory selling point, the ability to define the impact of one component on the perception of another is extremely valuable in improvement and development of products. As previously discussed (Chapter 3), literature studies indicate the modalities of taste and smell display both intra- and cross-modal interactions, and the preceding Chapter describes investigations exploring these interactions in detail in a citrus-flavoured model beverage system. The addition of carbonation adds another level of complexity in understanding flavour perception of a beverage bringing as it does, the modality of mouthfeel/texture to the forefront. Despite this, and the large consumption of carbonates, literature on the subject of carbonation and its impact on perceptual attributes of beverages are fairly limited. Studies have attempted to elucidate the mechanism by which addition of CO2 results in the bubbly or

fizzy mouthfeel perceived but few have focussed in depth on its impact on other modalities and associated attributes within beverages.

4.1.1. Mode of action of carbonation

The sensations experienced in the mouth due to carbonation are mediated by the trigeminal system and have been described as tingling, numbing, prickling, burning and even pain. Interestingly, the perception of carbonation in mouth is not due solely to the action of bubbles bursting activating the oral mechanoreceptors. A number of studies in the late 1990's and early 2000's reported the conversion of CO_2 to carbonic acid was implicit in the sensation of tingling associated with carbonation (Simons *et al.* 1999; Dessirier *et al.* 2000; Dessirier *et al.* 2001; Carstens *et al.* 2002). These studies used carbonic anhydrase inhibitors (dorzolamide or acetazolamide) to block the conversion of CO_2 to carbonic acid.

Both Simons (1999) and Dessirier (2000) report findings from human psychophysics studies using a split tongue paradigm. The carbonic anhydrase inhibitor was applied to one half of the tongue and subjects immersed the whole tongue in carbonated water and reported which side of the tongue had the strongest sensation. Analysis revealed both dorzolamide and acetazolamide were able to reduce the intensity of sensation elicited by carbonated water in a dose dependant manner. It would appear the use of carbonic anhydrase inhibitors significantly reduced the sensation attributed to CO_2 but did not fully eliminate the perception of 'fizziness'.

Furthermore, neither inhibitor affected the intensity of an acidic solution (pentanoic or citric acid) when applied in the same manner which would suggest the irritation elicited by acids is not carbonic anhydrase dependant. Tactile sensitivity of the tongue was not affected by application of the carbonic anhydrase inhibitors, suggesting an anaesthetic effect of the inhibitors was not responsible for the decrease in perceived sensation.

Dessirier *et al* (2000) and Simons *et al* (1999) used immunocytochemical measures of expression of an immediate early gene (c-fos) as a marker of neuronal activity in rat brain to show carbonated water administered to the surface of the tongue elicited activation within the trigeminal nerve system. Complementary experiments within the same studies, using electrophysiology, suggest this activation is a result of carbonic anhydrase dependant excitation by carbonated water of the oral nociceptors.

The chemogenic, rather than mechanical, mode of action of CO_2 suggested by these studies is in agreement with findings from McEvoy (1998), utilising a hyperbaric chamber to restrict the formation of bubbles. This study reported sensations of some mouthfeel attributes associated with CO_2 (tingling/prickly, pain/burn, throat-burn) were not rated as significantly different when scored in hyperbaric conditions (i.e. absence of bubbles) when compared to normal atmospheric conditions.

These studies provide convincing evidence that, at least some, sensations associated with CO_2 have a chemogenic rather than mechanical origin. It would appear therefore, that the sensations due to carbonation of products are derived from different mechanistic pathways and involve both oral mechanoreceptors and nociceptors, and consequential activation of the trigeminal nerve system. Activation of these receptors and the oral irritation stimulated may also have implications for other sensory attributes. Previous research has suggested that trigeminal system stimulation may influence both gustatory and olfactory systems (Bouvet *et al.* 1987; Cowart 1998; Brand 2006; Verhagen *et al.* 2006), however, few studies have used carbonated beverages to provide the trigeminal activation.

4.1.2. Taste-carbonation interactions

A small number of research groups have investigated the impact of carbonation on tastants, most studies finding little or no effect on sweetness but enhancement of sourness (McLellan *et al.* 1984; Comettomuniz *et al.*

1987; Yau *et al.* 1992; Cowart 1998; Odake 2001; Prescott *et al.* 2004). CO_2 alone is reported to have a sour taste when dissolved in water (Odake 2001). This has been attributed to the mix of carbonate, bicarbonate, carbonic acid and CO_2 at equilibrium which results in the presence of dissociated H^+ ions. Consequently, the enhancement of sourness may simply be the result of the presence of carbonic acid.

Anecdotal evidence would suggest carbonation suppresses sweetness; a 'flat' carbonated beverage is commonly reported to be sweeter than a 'fizzy' one. Nonetheless, results from published studies are inconsistent in demonstrating an effect of carbonation on sweetness perception. Evidence from McLellan *et al* (1984) using a carbonated apple juice beverage provides evidence of suppression of sweetness due to carbonation. However, subsequent investigations showed increasing carbonation did not significantly suppress sweetness of either sucrose (Comettomuniz *et al.* 1987; Yau *et al.* 1992), aspartame (Comettomuniz *et al.* 1987) or sodium cyclamate (Prescott *et al.* 2004).

However, Cowart (1998) investigated the effect of carbonation on gustatory stimuli (sweet, salty, sour and bitter) and reported a decrease in sweetness of sucrose by carbonation. The same study reported an increase in sourness with carbonation but no significant effect on saltiness or bitterness. Interestingly, analysis of binary combinations of the four tastants produced evidence suggesting the addition of carbonation did not significantly affect perceived sourness or sweetness, but did increase perception of bitterness, of a sucrose-citric acid mixture. Cowart went on to suggest CO_2 not only impacts on taste perception but this may manifest as altered taste quality rather than tastant intensity and that by limiting the number of taste categories that can be rated (as in earlier studies) these effects may be obscured.

4.1.3. Aroma-carbonation interactions

The published literature exploring the effect of carbonation on perception of aroma volatiles is minimal. While McLellan et al (1984) investigated interactions within a carbonated apple juice beverage; they report only on intensity of apple aroma and provide no ratings of apple flavour by mouth. Using a trained panel and Quantitative Descriptive Analysis, ratings for 'fruity apple aroma' were not significantly altered by carbonation and they concluded that the 'effervescence in the poured beverage did not significantly impinge on the effect of apple juice aroma volatiles present in the headspace'.

Two subsequent studies (Yau *et al.* 1989; Lederer *et al.* 1991), examined sensory properties in carbonated, flavoured milk beverages. The first of these (Yau *et al.* 1989) used a trained panel to assess blueberry flavoured milk drinks and concluded addition of CO_2 significantly increased blueberry flavour intensity. Lederer at al (1991) examined a range of flavoured milk products; strawberry, raspberry, peach and rootbeer, but only reported flavour intensity ratings for 'cooked milk' flavour which they determined was significantly suppressed by carbonation. However, the same study found carbonation-induced suppression of sweetness intensity, and although this was only significant in the raspberry flavoured product, it suggested a potential relationship between carbonation, flavour and sweetness perception.

There are two main draw-backs in relating these studies to standard carbonated beverages; firstly, milk is a very different system base compared to water, bringing a number of different, inherent sensory properties including flavour and mouthfeel attributes. Secondly, in both studies, the levels of carbonation were not comparable to those found in standard, commercial carbonated soft drinks. In the milk systems, levels were restricted to a maximum of 1.7 and 1.4vols CO_2 (Yau *et al.* 1989; Lederer *et al.* 1991) respectively), whereas common levels for standard beverages are in the

range 2.5-4vol CO_2. This disparity, together with restriction of response alternatives, may be responsible for the variable effects of carbonation on flavour-by-mouth attributes reported.

It is apparent that the interaction of carbonation with aroma/flavour is an area of research requiring much further study to ascertain perceptual impact of one on the other.

In the following investigations, a model beverage system, based on the design employed in previous studies into sugar-acid-aroma interactions (Chapter 3), was created using sugars (glucose and fructose, equi-sweet levels), citric acid and carbonation as design factors. Citric acid was the only acid examined as previous research suggested no significant differences in the effect of lactic and citric acid on perception of flavour, sweetness or sourness within this system. Furthermore, aroma volatiles (citral and limonene) were included at a constant level across the model designs as previous data suggested minimal significant impact of aroma on tastant perception (Chapter 3).

In this way, drawing on knowledge gained in prior experiments aliased to the present study, the number of design factors was reduced, limiting the number of samples required to explore the beverage system despite increasing its complexity by the addition of carbonation.

As the addition of carbonation to a beverage may well impact on many different sensory attributes, and previous studies have noted emergence of previously undetected attributes (Cowart 1998), the methodology for assessment was changed to allow broader evaluation.

Due to the importance of encompassing all perceived attributes within the scoring paradigm, the evaluation method of sensory profiling was chosen as being most appropriate. Use of this methodology has additional benefit in reducing errors associated with 'dumping' of sensations into inappropriate attribute ratings when response alternatives are limited (Lawless *et al.* 1992).

This 'dumping' effect may have been a limiting factor in initial investigations into the model system (Chapter 3) where only 3 attributes were rated (flavour, sweetness and sourness). Profiling methodology allows rating of assessor-generated attributes describing all sensory aspects discriminating between the model beverage samples.

4.2. Materials and Method

4.2.1. Sensory panel

A total of 10 assessors (2 male, 8 females, aged between 43-68yrs) from the University of Nottingham external sensory panel, were invited to take part in the study after completing appropriate screening tests for the samples under investigation. All assessors had prior experience of the test methodology used in this investigation (sensory profiling) and all but 1 had participated in the previous studies described in Chapter 3.

As new guidelines regarding use of individuals for sensory tests and constituents of samples under investigation had been introduced subsequent to the previous studies, full approval of the Ethics Committee at Nottingham University was sought and obtained before starting this study.

4.2.2. Design space

D-optimal designs (created in Design Expert software, Stat-Ease Inc, Minneapolis) were constructed using glucose (0-150g/l) or fructose (0-64g/l), and citric acid (0-1.5g/l) as numerical factors and carbonation as a categorical factor (nominally; none, low and high). Carbonation level was included as a categorical factor to allow use of carbonation levels which had been pre-determined to be perceivably different.

Volatile level (2.5ppm citral and limonene) was constant for all samples. Sugar and acid levels were chosen to be within the ranges found in commercially available beverages and ranges used have previously been

reported to be perceptually equi-sweet and equi-sour respectively (Savant *et al.* 2004). Two models were generated (Model G and Model F) and are described in Table 4-1. 18 samples from within the design space of each model (including 3 replicate and 3 lack of fit points) were used for evaluation of sensory attributes.

Table 4-1: Models 1 and 2 design limits

Design	Sugar	Acid	Aroma volatiles	Carbonation
Model G	Glucose (0-150g/l)	Citric acid (0-1.5g/l)	2.5ppm citral, 2.5ppm limonene	none, low (~1.5vols CO_2), high (~3.6volsCO_2)
Model F	Fructose (1-64g/l)	Citric acid (0-1.5g/l)	2.5ppm citral, 2.5ppm limonene	none, low (~1.5vols CO_2), high (~3.6volsCO_2)

4.2.3. Sample preparation and presentation

Design samples were made on the day of testing using mineral water (Brecon Carreg, UK), citric acid (99% Lancaster Synthesis, Lancaster, UK), lactic acid (Sigma, USA), glucose (Fisher Scientific, Loughborough, UK), and D(-)-fructose (98% Acros Organics, USA) as indicated by the model designs (Table 4-2 and Table 4-3). Aroma volatiles (citral and limonene, Aldrich, Dorset, UK), dissolved in propylene glycol (Fisher Scientific, Loughborough, UK), were added to obtain a final concentration of 2.5ppm citral and 2.5ppm limonene.

Table 4-2: Composition of samples in Model G design

Sample/ product	glucose (g/l)	citric acid (g/l)	CO_2 Level
1	150	1.5	none
2	0	0	high
3	150	1.5	high
4	0	1.5	none
5	75	0	low
6	75	1.5	low
7	150	0.75	low
8	0	0	none
9	150	0	high
10	0	0.75	low
11	0	1.5	high
12	75	0.75	none
13	150	0	high
14	37.5	0.75	high
15	75	1.5	low
16	150	0.75	low
17	0	0	low
18	150	0	none

Table 4-3: Composition of samples in Model F design

Sample/ product	fructose (g/l)	citric acid (g/l)	CO_2 Level
1	0	0	high
2	64	1.5	high
3	64	0.75	low
4	64	0.75	low
5	16	0.75	none
6	64	0	high
7	32	0	low
8	0	0	none
9	32	1.5	low
10	64	0	none
11	0	0.75	low
12	64	1.5	none
13	0	0	low
14	32	0.75	high
15	0	1.5	none
16	32	1.5	low
17	64	0	none
18	0	1.5	high

Samples were subsequently mixed on a roller bed for a minimum of 1h to ensure all components were fully dissolved and dispersed. All samples were refrigerated (4-6°C) prior to carbonating.

4.2.3.1. Carbonation of samples

Carbonation of samples was performed as previously described (Chapter 2 section 2.2.2.1.1). Briefly; samples were carbonated by setting the delivered CO_2 gas pressure to the desired level, opening the isolation switch and shaking the sample bottle to speed the dispersion of CO_2 into the sample. Once equilibrium was achieved, the sample bottle was isolated and the pressure within the bottle monitored using the second pressure gauge to ensure the correct pressure had been delivered and was maintained. The sample was then removed from the carbonating apparatus and aliquoted into small, glass, screw-topped vials (35mls) as quickly as possible before refrigeration storage (4-6°C). Each vial was filled to the brim to reduce loss of CO_2 into headspace.

Temperature plays an important role in regulating the amount of CO_2 dissolved in solution; the lower the temperature, the more CO_2 can be dissolved. As carbonated beverages are commonly consumed at temperatures between 0-10°C, the samples in this study were stored at 4-6°C prior to and following carbonation. In the design of these studies, carbonation was required at three categorical levels (Table 4-1). After some preliminary testing, the three levels chosen were 0, 1.5vols and 3.6vols of CO_2. These levels were identified as being perceivably different in terms of fizziness and are within the ranges commonly found in soft drink beverages. Most commercially available carbonated drinks contain between 2-4vols CO_2 (fruit flavoured carbonated beverages being generally lower in carbonation level then cola flavoured ones).

Although samples were accurately carbonated to these predetermined levels, some carbonation was unavoidably lost on removal of the sample from the

carbonating apparatus, therefore these levels are subsequently referred to as none, low and high carbonation.

4.2.3.2. Sample presentation

Samples (35ml) were presented in identical clear glass vials fitted with a screw top lid, each labelled with a randomly generated 3 digit code, in a randomised, balanced order across the panellists. Samples were presented monadically, in sets of 3, with breaks of 15 minutes between sets. Samples were removed from refrigeration (4-6°C) no more than 5mins before presentation for assessment. A minimum of 1min was allowed before presenting the next sample in the set of 3 to ensure no carry-over effects.

4.2.4. Sensory evaluation

A method of sensory profiling was used to enable quantification of all attributes of the model system (ISO 6564-1985, BS 5929: Part 4: 1986 Sensory analysis – Methodology – Flavour profile methods). This method involves assessors generating descriptors/attributes which they feel fully describe and discriminate between the samples and using these to rate each sample thus allowing comparison of samples with different composition.

4.2.4.1. Generation of attributes

Samples taken from within the design space of Model G, reflecting the extremes of all components, were used for generation of attributes. In sensory booths, assessors were given each sample monadically and asked to describe it under headings of 'visual', 'aroma', 'mouthfeel', 'flavour and taste', 'aftertaste' and 'other'. Subsequently, the whole panel was gathered in the training room and descriptors used were discussed. Only attributes which, by consensus, the panel agreed described and discriminated between the samples were used. Each term was fully defined and explained to remove any doubt about its meaning. The use of scale was discussed and verbal 'anchors' for scale ends agreed upon.

4.2.4.2. *Agreement and training on attributes*

Several sessions were then devoted to familiarisation with attributes, procedures for assessment, scales and rating. A protocol for assessment of each attribute was defined, with agreement of the assessors, to ensure all were evaluating samples in the same way (Table 4-4).

Table 4-4: Test protocols used for assessment of attributes

CATEGORY	ATTRIBUTE	PROTOCOL
MOUTHFEEL	overall impression of fizziness	*Hold sample in mouth for 10 seconds whilst evaluating the 'overall fizziness', 'tingling' and 'drying' attributes, then swallow. Rate the 'irritant' sensation at its strongest intensity (this may be immediately after swallowing)*
	tingling	
	drying/astringent	
	Irritant/burning (chemical)	
FLAVOUR AND TASTE	citrus-like flavour	*Hold the sample in mouth for 10 seconds before swallowing, ensure sample fully coats mouth fully including sides and back of tongue*
	sweetness	
	sourness	
AFTERTASTE	bitter aftertaste	*Hold the sample in mouth for 10seconds, swallow, after a further 5 seconds rate the aftertaste attributes*
	acidic aftertaste	
	drying aftertaste	

Reference samples, where available, were provided which exemplified the attribute in question, eg glucose (sweetness), citric acid (sourness), aroma volatiles (citrus flavour), carbonated water (fizziness, tingling and irritant). The same subset of samples used for generation of attributes was used for these training sessions. Panellists scored each agreed attribute in isolated sensory booths with access to a table of attribute definitions and testing

protocols, using a computerised data capture system Fizz (Biosystemes, France).

Attributes were categorised according to rating protocol used (Table 4-4), and these categories nominally termed 'mouthfeel', 'taste/flavour' and 'aftertaste'. Ratings were made on a horizontal line scale with agreed scale anchor terms at each end. After rating, panellists were given feedback regarding the group performance. Attributes showing inconsistency were re-examined by the group, using the same samples, until a consensus was reached.

4.2.4.3. Assessment of samples

All testing was performed at room temperature in an air-conditioned room, under Northern Hemisphere daylight and in individual booths. Data were collected using the computerised data acquisition system, Fizz (Biosystemes, France).

For each model, 18 samples indicated by the D-optimal design were rated for all attributes by assessors. Sample composition for Model G (glucose/citric acid/carbonation) and Model F (fructose/citric acid/carbonation) are shown in Tables 4-2 and 4-3 respectively.

Samples were presented monadically, in sets of 3, with breaks of 15 minutes between sets. Panellists were instructed to consume the sample as indicated by the assessment protocol (Table 4-4), score each attribute by means of placing a tick on a computerised line scale (Fizz, Biosystemes, France) and palate cleanse using cracker and water between samples. All 18 samples were evaluated per session and 3 sessions were used to allow for 3 replicate measures.

4.2.5. Data analysis and panel performance monitoring

Panel performance was monitored by assessment of replicate scores. A repeatability index was calculated by Fizz sensory software (Biosystemes, France) using coefficient of variance (CV) values subjected to Analysis of Variance (ANOVA) as described in Chapter 3 (section 3.2.6).

Assessment indicated individual panellist's precision (repeatability of replicate data) and ability to discrimination between samples (p value obtained by one way ANOVA by product for each individual).

Principal component analysis (PCA) constructs linear combinations (where 2nd and subsequent principal components are orthogonal to each other) of the original variables that account for the maximal variance in the data. PCA was performed on the mean panel data to identify the key attributes contributing the most variation in samples within the design space. To simplify the interpretation of factors, by minimizing the number of variables that contribute significantly to each factor, varimax rotation was used (XLSTAT version 7.5.2, Addinsoft, USA).

The goal of this orthogonal varimax rotation is to identify a factorial structure where for each factor, a few variables have strong contributions and the other factors have very weak contributions. This goal is obtained by maximizing, for a given factor, the variance of the squares of the contributions among the variables, with the constraint that the variance of each variable must remain unchanged (XLSTATPro Help version 7.5.2).

Two-way ANOVA (analysis by attribute with product and judge factors) of the mean panel data was performed to identify significant differences between the samples within each Model design, for each of the attributes assessed. Subsequently, where appropriate, Tukey's HSD multiple comparison tests were performed to determined which samples differed significantly for rated intensity of each attribute.

4.2.5.1. Generation of Predictive Models

Predictive polynomial models were generated to explain variations in perception of each attribute as a function of sugar, acid and carbonation levels. As detailed previously (Chapter 3, section 3.3.3), non-significant terms, as determined by ANOVA, were removed and a final mathematical model was chosen which best represented the data after scrutiny of best-fit equations and associated model values. The predictive ability of these models was assessed by means of the evaluation of a separate set of samples taken from within the design space but not part of the original model data.

4.3. Results

4.3.1. Generation of attributes

After lengthy discussion and retesting of samples, the panel reached a consensus agreement on a lexicon to describe the attributes associated with the beverage samples. Thorough deliberation with panellists enabled clear delineation between descriptor definitions to prevent overlap between attribute meanings and possible subsequent misinterpretation in allocation of scores. Attributes, definitions and scale line anchors are listed in Table 4-5.

During the training sessions, panellists practised scoring these attributes using the agreed testing protocols (Table 4.4). Panellists were able to score attributes consistently and attribute mean scores across the panel suggested agreement within the group with regard to the specific sensation (attribute) rated.

Table 4-5: Panel generated attributes, agreed definitions and scale anchors

ATTRIBUTE	DEFINITION	SCALE
overall impression of fizziness	*overall perception in the whole mouth including both bubbling feeling and pain perception*	LOW - - - HIGH
tingling	*sensation associated with fizz/acidity on tongue and around inside of mouth – like a pins and needles sensation*	LOW - - - HIGH
drying/astringent	*tactile sensation due to shrinking, drawing or puckering of oral epithelium*	NOT- - - VERY
irritant/burning (chemical)	*Irritating/burning sensation which lingers after the stimulus is removed*	LOW - - - HIGH
citrus-like flavour	*lemon/lime/citrus flavour*	LOW - - - HIGH
sweetness	*taste stimulated by sugar in water*	NOT- - - VERY
sourness	*taste stimulated by acid in water*	NOT- - - VERY
bitter aftertaste	*aftertaste stimulated by caffeine in water*	NOT- - - VERY
acidic aftertaste	*aftertaste stimulated by acid in water*	NOT- - - VERY
drying aftertaste	*aftertaste tactile sensation due to shrinking, drawing or puckering of oral epithelium*	NOT- - - VERY

4.3.2. Panel performance monitoring

Measures of repeatability and discriminative ability of the panellists were used to assess panel performance. Analysis of coefficient of variance (CV)

data calculated for each panellist suggested all panellists were able to score most attributes reliably across the 3 sessions.

One-way ANOVA performed on this data demonstrated the ability of panellists to discriminate between samples in terms of the agreed attributes. Assessment of probability values (FPROD) revealed the majority of the panel could reliably discriminate between samples for the majority of attributes.

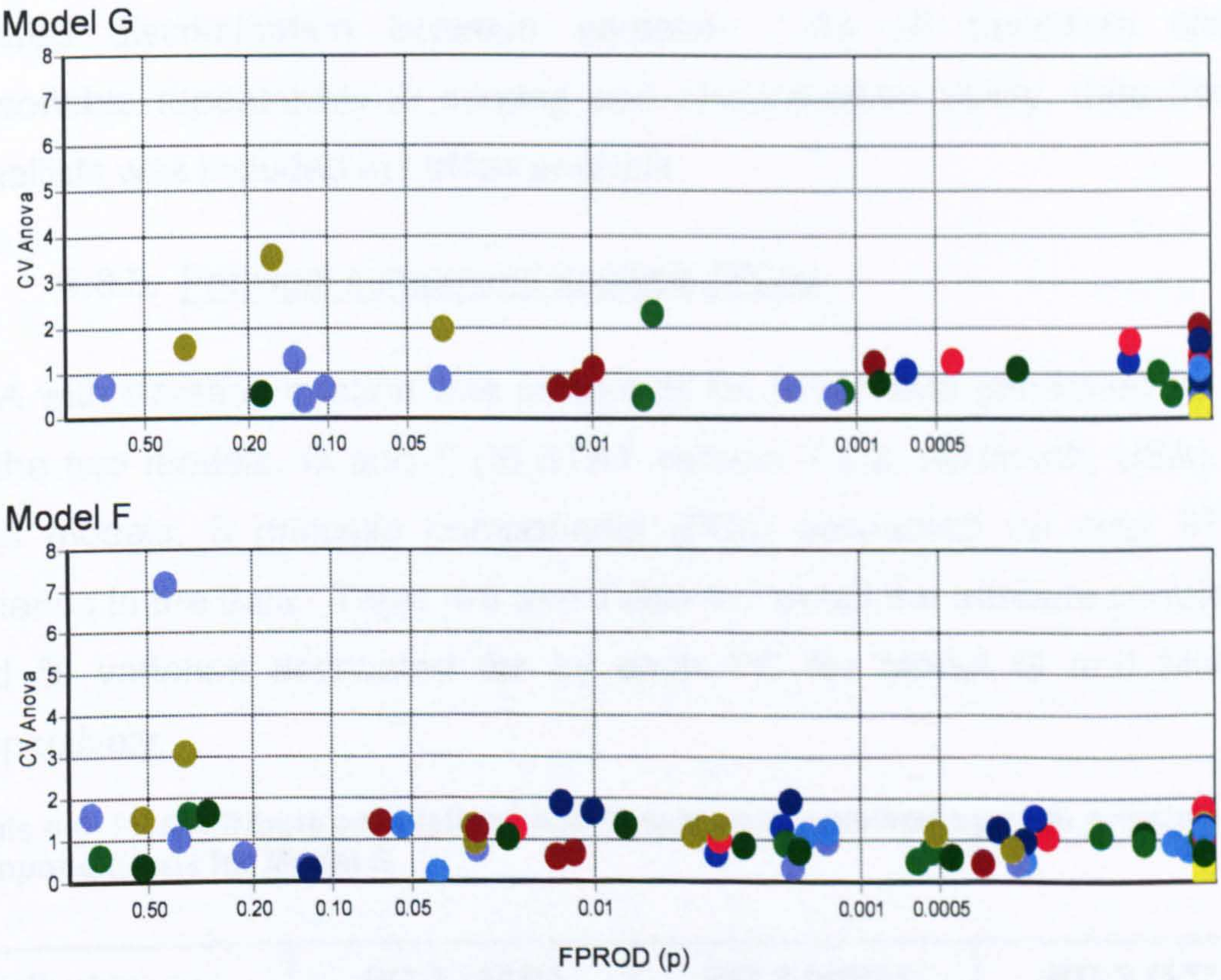


Figure 4-1: Panel monitoring; repeatability and discrimination.

Panel monitoring data for Models G and F showing coefficient of variance (CV) plotted against discrimination probability (FPROD). Data points are colour coded for attributes and each data point represents a panellist's mean result (3 replicates)

It should be noted that 4 panellists were unable to discriminate (significant at $p<0.1$) between samples on scoring of the attribute 'citrus flavour'. A further 2 panellists were also unable to significantly discriminate ($p<0.1$) between

samples on the basis of bitter aftertaste. The level of aroma volatiles (citral and limonene) was constant for all the samples examined, therefore some difficulty in discrimination of this attribute is to be expected. That most panellists are able to discriminate between samples significantly at the 10% confidence level is encouraging and suggests interactions occurring within the model systems were influencing flavour perception.

Rating of all other attributes by panellists yielded reproducible data which allowed discrimination between samples. As all panellists showed reasonable repeatability in scoring and discriminative ability, data from all panellists was included in further analysis.

4.3.3. Principal component analysis (PCA)

PCA with Varimax rotation was performed on panel data generated for each of the two models, G and F (XLSTAT version 7.5.2, Addinsoft, USA). For both models, 3 principle components (PCs) accounted for over 97% of variance in the data. Table 4-6 and Table 4-7 detail the attribute correlations and % variance accounted for by each PC for Model G and Model F respectively.

Table 4-6: PCA attribute correlations and % variance explained by each principle component axis for Model G

Attributes	PC 1 (45%)	PC 2 (40%)	PC 3 (13%)
fizziness	**0.967**	-0.211	-0.120
tingling	**0.962**	-0.220	-0.156
drying	**0.621**	**-0.770**	-0.083
irritant	**0.964**	-0.228	-0.027
citrus flavour	-0.210	-0.153	**0.849**
sweetness	0.087	**0.721**	**0.684**
sourness	0.085	**-0.990**	0.065
bitter AT	0.513	**-0.745**	-0.334
acidic AT	0.274	**-0.941**	0.154
drying AT	0.504	**-0.853**	-0.014

Table 4-7 PCA attribute correlations and % variance explained by each principle component axis for Model F

Attributes	PC 1 (47%)	PC 2 (23%)	PC 3 (27%)
fizziness	**0.948**	-0.135	0.271
tingling	**0.938**	-0.169	0.295
drying	**0.623**	-0.318	**0.698**
irritant	**0.924**	-0.194	0.318
citrus flavour	-0.242	**0.778**	-0.112
sweetness	0.036	**0.934**	-0.336
sourness	0.233	-0.324	**0.905**
bitter AT	0.529	**-0.730**	0.311
acidic AT	0.353	-0.173	**0.915**
drying AT	0.600	-0.354	**0.696**

Examination of PC correlation values suggest for both Models G and F, suggested that PC1 described the mouthfeel attributes (fizziness, tingling, drying in mouth and irritant). Some differences between the two models can be identified with regard to PC's 2 and 3. In Model G, PC2 is described by sweetness and sourness, along with the aftertaste attributes (bitter, acidic and drying) whilst in Model F sourness, acidic and drying aftertastes correlate highly with PC3. Citrus flavour and sweetness are highly correlated with PC3 in Model G, whereas these attributes correlate with PC2 in Model F. It should be noted, however, that in Model F the % variance explained by PC3 is higher than PC2 (27% compared to 23%), as a consequence of the varimax rotation applied. Therefore in both models, the PC correlated with sourness and aftertastes accounts for more variance than the PC correlated with sweetness and citrus flavour.

These relationships can be clearly seen by examination of bi-plots displaying both attributes and samples. Bi-plots describing the sample loadings on the PCs are shown in Figure 4-2 (PC1and 2) and Figure 4-3 (PC1 and 3) for Model G, and Figure 4-4 (PC1 and 2) and Figure 4-5 (PC1 and 3) for Model F. The inset plots show the sample spread without attribute vectors for

easier identification. These showed analysis of panel scores for duplicate samples included in the model designs resulted in almost identical loading on PCs as expected (e.g samples 9 and 13, Model G, Figure 4-2). Interpretation of the bi-plots for Models G and F yielded similar findings; therefore, whilst plots from both Models are included for comparisons, discussion will be centred on Model G.

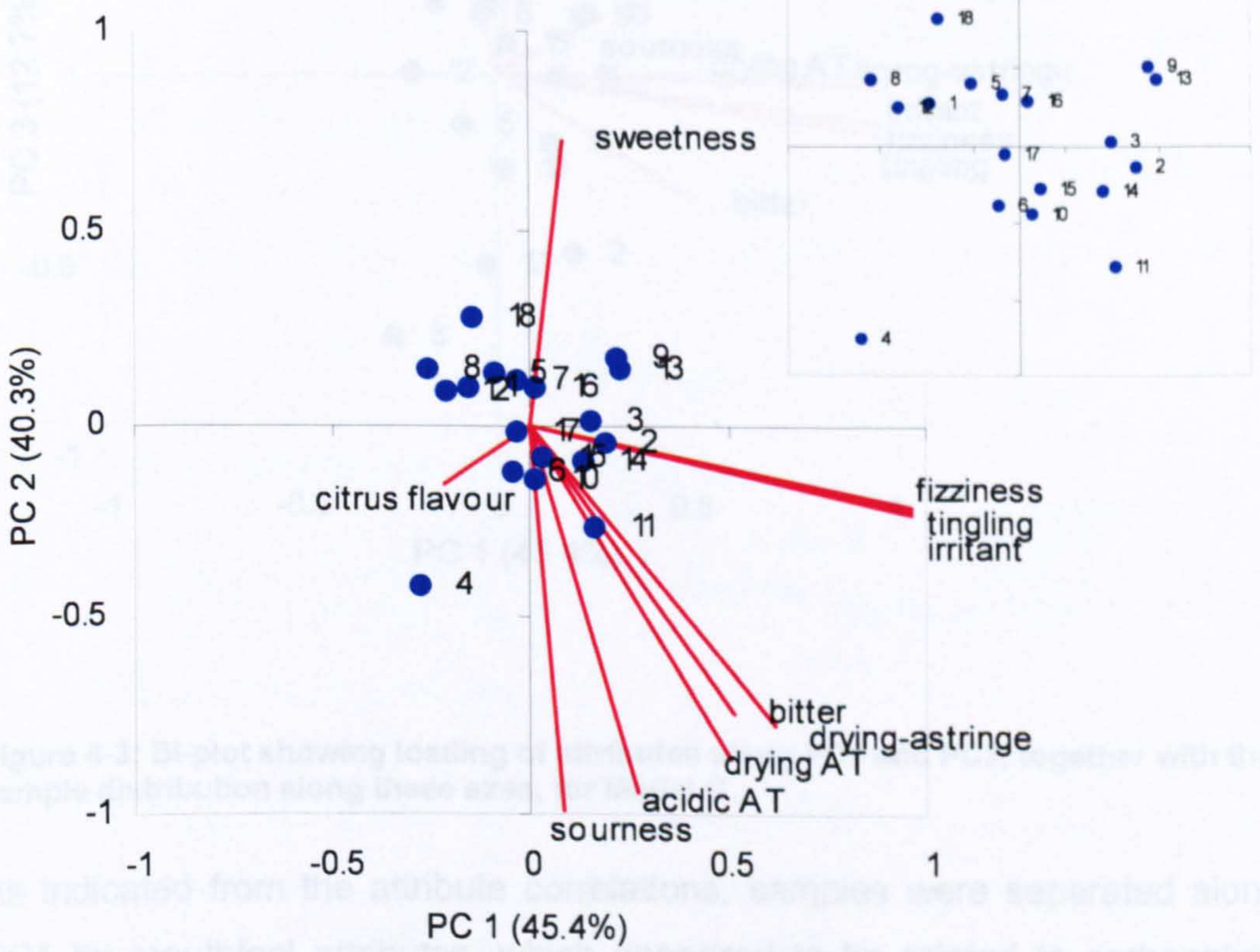


Figure 4-2: Bi-plot showing loading of attributes along PC1 and PC2, together with the sample distribution along these axes, for Model G.

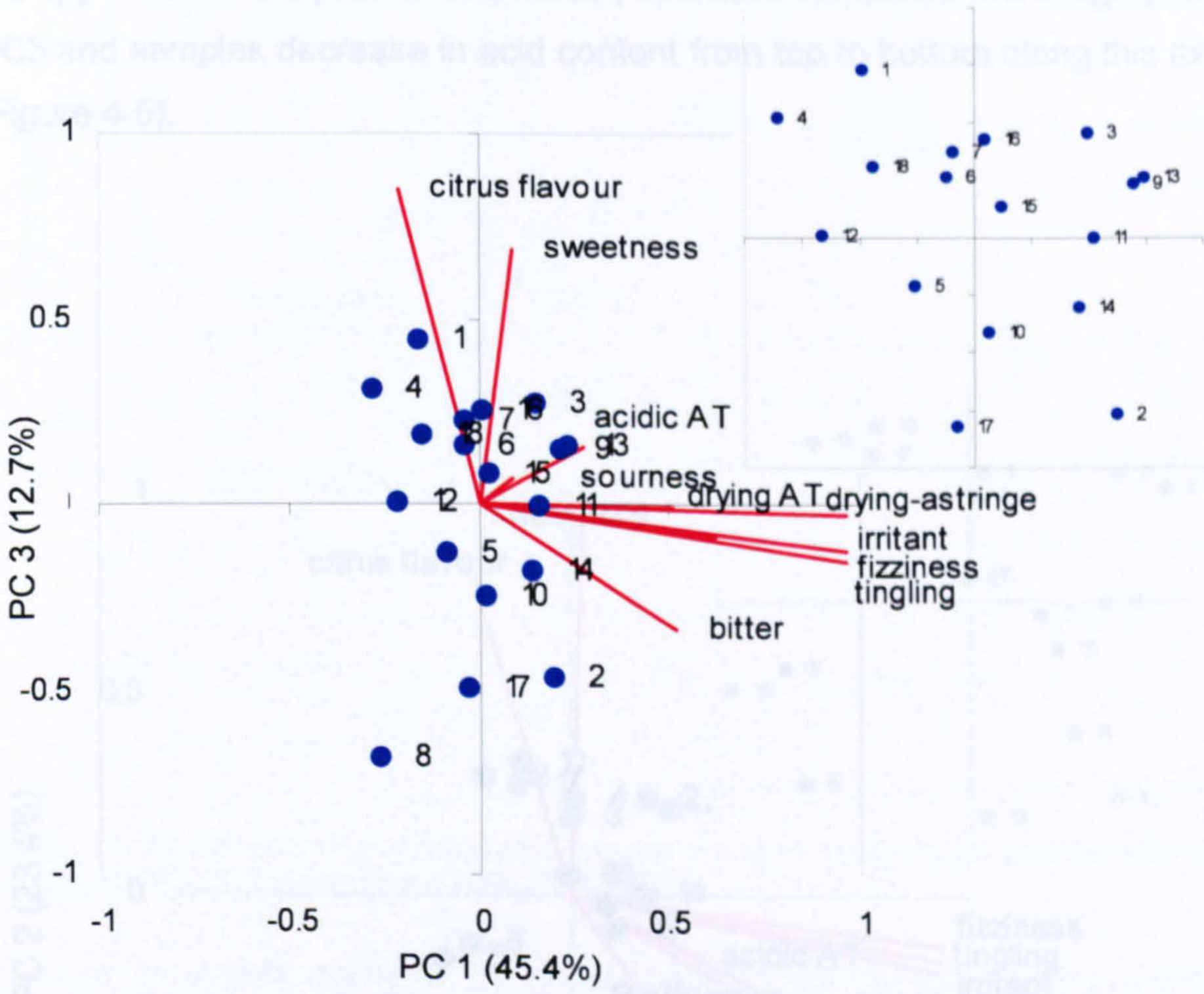


Figure 4-3: Bi-plot showing loading of attributes along PC1 and PC3, together with the sample distribution along these axes, for Model G.

As indicated from the attribute correlations, samples were separated along PC1 by mouthfeel attributes, which appeared to be related to carbonation level. In Figure 4-2 and Figure 4-4, samples not carbonated tended to be distributed in the top left hand side of the plots, those with low carbonation in the centre portion, and those with high carbonation in the bottom right hand portion. In Model G, both sweetness and sourness attributes dominate the sample separation along PC2. Figure 4-2 shows a trend linked to sample composition; samples tend to increase in glucose content from the top to bottom halves of the plot and increase in acid content from bottom to top. In

Model F, this trend for samples to have increasing fructose content along PC2 is even clearer with all samples with higher fructose content contained in the upper half of the plot. In this model, sourness correlated more highly with PC3 and samples decrease in acid content from top to bottom along this axis (Figure 4-5).

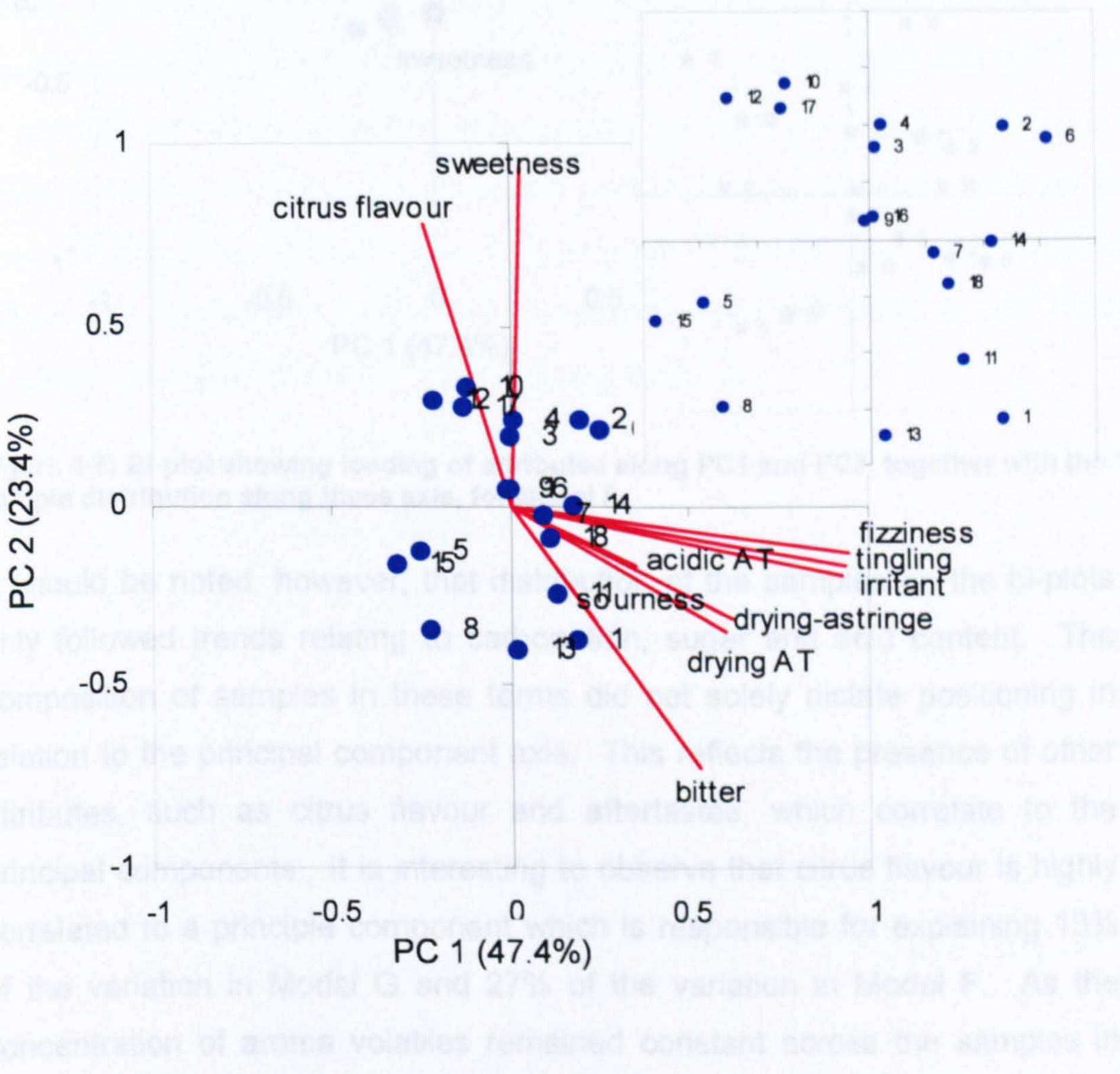


Figure 4-4: Bi-plot showing loading of attributes along PC1 and PC2, together with the sample distribution along these axes, for Model F.

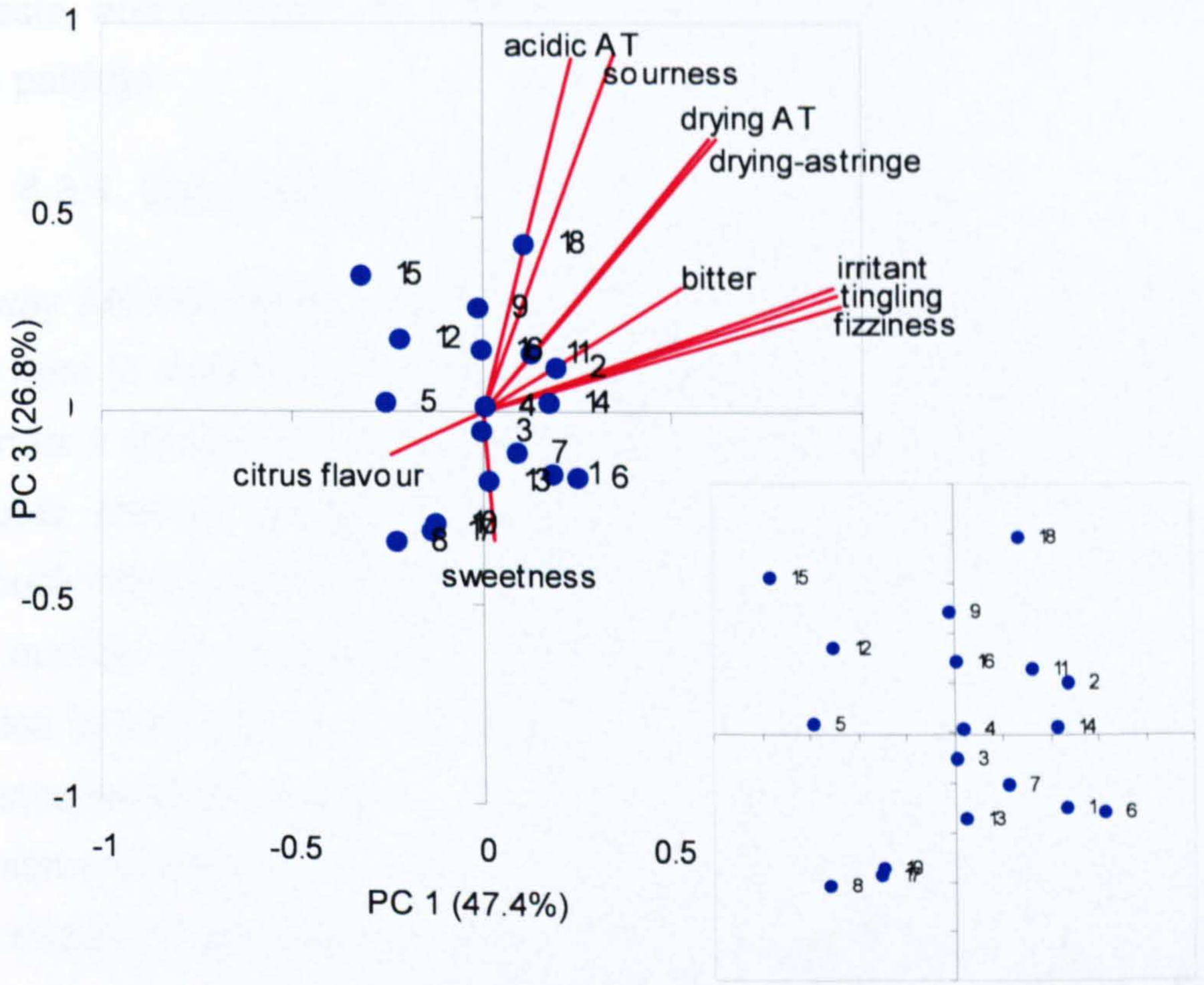


Figure 4-5: Bi-plot showing loading of attributes along PC1 and PC3, together with the sample distribution along these axis, for Model F.

It should be noted, however, that distribution of the samples on the bi-plots only followed trends relating to carbonation, sugar and acid content. The composition of samples in these terms did not solely dictate positioning in relation to the principal component axis. This reflects the presence of other attributes, such as citrus flavour and aftertastes, which correlate to the principal components. It is interesting to observe that citrus flavour is highly correlated to a principle component which is responsible for explaining 13% of the variation in Model G and 27% of the variation in Model F. As the concentration of aroma volatiles remained constant across the samples in both systems, this would suggest other elements are able to influence perception of citrus flavour within these samples.

Examination of correlations between attributes showed attributes fizziness, tingling and irritant were highly correlated with each other as indicated by

their loadings on the bi-plots. The attribute pairs drying in-mouth and drying aftertaste, and sourness and acidic aftertaste also showed high correlation within pairings.

4.3.4. Data analysis

Two-way ANOVA (judge and product factors) was performed on the mean panel data (3 replicates) for each model. Full ANOVA tables are included in Appendix 2 (Model G) and 3 (Model F). ANOVA data from a number of attributes showed significant judge and product-judge interactions. As previously discussed (Chapter 3, section 3.3.1), this is attributable to the large number of samples within each model design, the similarity of many samples in terms of assessed attributes and the absence of an appropriate reference leading to differences in scale usage. The panel mean, standard deviation and results of multiple comparison analysis of Model G and Model F are shown in Table 4-8 and Table 4-9 respectively.

Table 4-8: Model G (glucose) mean panel scores (standard deviation) and post hoc test groupings

	Attribute																	
	Fizziness		Tingling		Drying-astringent		Irritant		Citrus flavour		Sweetness		Sourness		Bitter aftertaste		Acidic aftertaste		Drying aftertaste	
sample	mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev	mean	stdev
1	0.24 ^{E}	0.11	0.32 ^{H}	0.52	1.74 ^{FGH}	1.32	2.02 ^{FGH}	3.3	6.82 ^{A}	2.63	7.79 ^{B}	1.08	2.46 ^{GHI}	1.64	0.89 ^{IJ}	0.56	2.98 ^{D}	1.4	2.36 ^{HIJ}	1.49
2	8.03 ^{A}	1.80	8.39 ^{A}	1.17	6.76 ^{A}	2.2	5.86 ^{A}	2.94	2.97 ^{H}	2.01	0.41 ^{FG}	0.41	4.26 ^{DEF}	2.45	4.57 ^{AB}	2.8	4.49 ^{BC}	2.14	6.16 ^{ABC}	1.94
3	6.67 ^{AB}	1.59	6.42 ^{BCD}	1.52	5.05 ^{BC}	2.02	5.09 ^{ABC}	2.74	5.98 ^{ABCD}	2.41	5.97 ^{C}	1.63	3.61 ^{FGH}	1.96	2.63 ^{DEFGH}	1.64	4.97 ^{BC}	1.32	5.06 ^{CDEF}	2.29
4	0.13 ^{E}	0.14	0.2 ^{H}	0.23	6.03 ^{AB}	2.13	1.46 ^{GHI}	1.57	6.15 ^{ABC}	2.83	0.24 ^{FG}	0.17	8.79 ^{A}	1.52	4.05 ^{ABCD}	3.09	7.74 ^{A}	1.62	7.06 ^{AB}	2.41
5	2.17 ^{D}	1.29	2.37 ^{G}	1.9	2.27 ^{FG}	1.43	2.17 ^{EFGH}	1.95	4.52 ^{DEFG}	2.64	4.58 ^{D}	1.35	1.51 ^{IJK}	1.15	1.79 ^{EFGHIJ}	1.77	2.39 ^{DE}	1.25	3.02 ^{GHI}	2.7
6	3.78 ^{CD}	0.91	3.85 ^{EFG}	1.15	4.76 ^{BCD}	1.29	3.31 ^{DEF}	1.72	6.43 ^{AB}	2.57	2.83 ^{E}	1.01	5.16 ^{CD}	1.51	2.96 ^{CDEF}	1.72	5.59 ^{BC}	1.29	4.96 ^{CDEF}	2.18
7	2.77 ^{D}	1.05	2.77 ^{FG}	1.02	2.81 ^{EF}	0.99	2.98 ^{DEFG}	2.37	5.59 ^{ABCDE}	2.34	6.92 ^{BC}	2.09	2.34 ^{GHIJ}	1.81	1.67 ^{FGHIJ}	1.49	2.97 ^{D}	1.66	3.1 ^{GHI}	1.68
8	0.07 ^{E}	0.12	0.09 ^{H}	0.16	1.07 ^{GH}	1.73	0.36 ^{I}	0.56	3.65 ^{FGH}	3.11	0.14 ^{G}	0.19	1.02 ^{JK}	2.16	1.3 ^{GHIJ}	1.7	1.1 ^{EF}	2.07	1.57 ^{IJ}	2.25
9	7.21 ^{A}	2.21	6.97 ^{ABC}	2.04	4.29 ^{CDE}	1.86	5.39 ^{AB}	3.15	4.81 ^{CDEFG}	3.05	7.71 ^{B}	1.19	1.79 ^{IJK}	1.58	2.85 ^{CDEFG}	2.45	2.92 ^{D}	1.44	4.15 ^{EFG}	2.22
10	5.3 ^{BC}	1.83	5.08 ^{DE}	1.98	5.79 ^{ABC}	2.19	4.21 ^{BCD}	2.51	3.63 ^{FGH}	2.7	0.36 ^{FG}	0.3	5.67 ^{C}	2	4.28 ^{ABC}	3.03	5.72 ^{BC}	2.33	5.47 ^{CDE}	2.43
11	7.96 ^{A}	1.78	8.07 ^{AB}	1.41	7.25 ^{A}	1.84	6.15 ^{A}	2.86	4.86 ^{CDEF}	1.79	0.46 ^{FG}	0.32	7.17 ^{B}	1.2	4.99 ^{A}	2.79	8.02 ^{A}	0.48	7.42 ^{A}	1.67
12	0.24 ^{E}	0.28	0.17 ^{H}	0.15	1.67 ^{FGH}	1.39	0.72 ^{HI}	0.82	5.71 ^{ABCD}	2.33	4.44 ^{D}	1.19	2.34 ^{GHIJ}	1.52	1.1 ^{HIJ}	0.54	2.51 ^{DE}	1.48	1.99 ^{IJ}	2.14
13	7.24 ^{A}	1.77	7.39 ^{AB}	1.58	4.73 ^{BCD}	2.01	6.02 ^{A}	3.24	4.94 ^{BCDEF}	2.42	7.37 ^{B}	1.65	2.28 ^{HIJ}	1.96	2.87 ^{CDEFG}	2.31	3.15 ^{CD}	1.49	4.47 ^{DEFG}	2.32
14	7.52 ^{A}	2.36	7.17 ^{AB}	2.31	5.85 ^{ABC}	2.28	5.28 ^{ABC}	3.54	4.56 ^{DEFG}	2.28	1.31 ^{FG}	0.59	5.03 ^{CDE}	1.49	4.22 ^{ABCD}	2.01	5.61 ^{B}	1.22	5.84 ^{BCD}	1.92
15	5.00 ^{CD}	1.73	5.17 ^{CDE}	1.74	4.82 ^{BCD}	2.02	3.72 ^{CDE}	1.83	5.5 ^{ABCDE}	2.07	3.12 ^{E}	1.12	4.53 ^{CDEF}	1.52	3.36 ^{BCDE}	1.92	5.68 ^{B}	0.81	5.49 ^{CDE}	1.41
16	3.83 ^{CD}	1.56	3.46 ^{EFG}	1.41	3.21 ^{DEF}	1.27	2.96 ^{DEFG}	2.22	5.61 ^{ABCDE}	2.42	7.12 ^{B}	1.44	2.39 ^{GHIJ}	2.17	2.24 ^{EFGHI}	1.73	3.3 ^{CD}	1.51	3.73 ^{FGH}	2.04
17	3.65 ^{CD}	1.19	4.4 ^{EF}	1.98	4.39 ^{BCDE}	2.39	3.74 ^{CDE}	2.26	3.28 ^{GH}	2.66	0.31 ^{FG}	0.24	3.68 ^{EFG}	2.44	4.77 ^{AB}	2.24	3.31 ^{CD}	2.41	4.54 ^{DEFG}	2.7
18	0.09 ^{E}	0.09	0.11 ^{H}	0.1	0.52 ^{H}	0.9	1.26 ^{HI}	2.02	4.1 ^{EFGH}	2.94	9.21 ^{A}	0.78	0.64 ^{K}	0.99	0.44 ^{J}	0.93	0.79 ^{F}	0.89	0.97 ^{J}	1.52

Samples assigned the same letter, within a column, are not significantly different

Table 4-9: Model F (fructose) mean panel scores (standard deviation) and post hoc test groupings

	Attribute									
	Fizziness	Tingling	Drying-astringent	Irritant	Citrus flavour	Sweetness	Sourness	Bitter aftertaste	Acidic aftertaste	Drying aftertaste
Sample	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
1	6.16 1.84 A	6.77 2.18 A	6.38 1.80 ABC	6.06 2.46 AB	3.31 2.14 D	0.59 0.48 E	3.85 2.39 DEFG	6.29 2.34 A	4.06 2.28 CDE	5.83 2.53 ABCD
2	6.55 1.74 A	6.88 1.63 A	5.70 1.48 BCD	5.85 2.20 AB	6.23 2.54 A	5.32 1.97 B	4.15 1.87 CDEF	3.33 1.29 BCDE	5.72 1.23 B	5.96 1.53 ABCD
3	2.85 1.26 B	3.38 1.65 C	3.62 1.51 EF	3.26 2.09 D	5.73 2.65 ABC	5.91 1.52 B	2.70 1.56 FGH	2.70 1.93 CDEF	3.61 1.59 DE	4.03 2.18 DEF
4	3.19 1.64 B	3.58 2.15 C	4.14 1.71 DEF	3.56 1.82 D	6.34 2.44 A	5.98 1.40 B	3.00 1.85 EFGH	2.56 1.37 DEFG	3.89 1.90 CDE	4.52 2.09 CDE
5	0.13 0.15 C	0.15 0.14 D	2.75 1.37 FG	1.22 1.81 E	5.81 2.42 ABC	1.86 1.35 D	3.48 1.88 EFG	3.34 1.94 BCDE	3.63 1.65 DE	3.76 2.08 EF
6	6.83 2.00 A	6.60 2.48 AB	4.56 1.70 CDEF	5.59 2.76 ABC	6.13 2.52 AB	6.37 2.13 B	2.36 2.18 GHI	3.29 1.75 BCDE	3.59 2.44 E	5.09 2.48 BCDE
7	4.02 1.62 B	4.80 1.91 BC	4.56 1.66 CDEF	4.86 1.92 ABCD	5.67 2.44 ABC	3.85 1.68 C	3.05 1.88 EFG	4.42 1.95 ABCD	3.54 1.95 E	5.06 2.44 CDE
8	0.12 0.22 C	0.19 0.34 D	1.14 1.36 GH	0.56 0.67 E	5.86 3.29 ABC	0.21 0.34 E	1.31 2.24 HI	2.46 2.51 EFG	1.43 2.27 FG	2.34 2.75 FG
9	3.87 1.33 B	4.53 1.99 C	6.18 1.66 ABC	4.35 2.05 BCD	5.91 2.80 ABC	2.93 1.55 CD	5.38 1.34 BCD	4.49 1.96 ABCD	5.97 1.39 B	6.15 2.18 ABC
10	0.19 0.23 C	0.09 0.10 D	0.76 1.09 H	0.78 0.84 E	6.16 2.69 AB	8.38 0.93 A	0.75 1.28 I	0.88 1.69 FG	0.80 1.24 G	1.19 1.83 G
11	6.26 2.06 A	6.71 2.20 AB	6.93 1.85 AB	6.10 2.17 AB	4.12 1.90 BCD	0.56 0.64 E	5.94 2.34 BC	6.13 2.11 A	5.89 2.15 B	6.98 1.95 AB
12	0.34 0.40 C	0.37 0.46 D	3.83 2.41 DEF	1.20 1.28 E	6.41 3.00 A	6.07 1.22 B	3.50 2.10 EFG	2.00 1.27 EFG	4.13 1.87 BCDE	4.06 2.35 DE
13	3.39 1.59 B	3.92 1.89 C	4.79 1.58 CDE	4.08 1.82 CD	3.91 2.75 CD	0.42 0.43 E	3.22 2.27 EFGH	6.15 2.37 A	3.18 2.44 EF	5.29 2.13 BCDE
14	6.24 2.10 A	6.57 2.25 AB	6.20 1.68 ABC	5.45 2.60 ABC	4.84 2.01 ABCD	3.62 1.81 C	4.48 1.23 BCDEF	4.58 0.95 ABC	4.60 1.57 BCDE	5.86 1.80 ABCD
15	0.11 0.14 C	0.28 0.44 D	4.92 1.17 CDE	1.40 1.69 E	4.47 2.52 ABCD	0.26 0.27 E	5.91 1.30 B	3.44 1.59 BCDE	5.59 1.59 BC	5.11 1.49 BCDE
16	3.84 2.02 B	4.02 1.48 C	5.07 1.34 BCDE	4.41 1.76 BCD	5.89 2.75 ABC	3.47 2.07 C	4.54 1.35 BCDE	4.30 0.81 ABCD	5.40 1.39 BCD	5.80 1.95 ABCD
17	0.09 0.08 C	0.07 0.06 D	0.73 0.93 H	1.01 1.20 E	5.68 2.70 ABC	7.78 1.60 A	0.78 1.31 I	0.61 0.91 G	0.75 1.25 G	1.28 1.65 G
18	7.01 1.36 A	7.78 1.42 A	7.74 1.77 A	6.25 2.10 A	4.52 1.92 ABCD	0.41 0.49 E	8.12 1.28 A	4.64 2.48 AB	7.93 1.10 A	7.17 1.80 A

Samples assigned the same letter, within a column, are not significantly different

4.3.5. Predictive models

Using the global mean of the panellists, significant polynomial predictive models were generated using multiple linear regression (Design Expert), which described the perceptual results in terms of the design factors used in each experiment (sugar [glucose or fructose], citric acid, carbonation) for each attribute assessed.

Due to the complexity of the model designs and the number of attributes assessed, a large number of predictive equations were generated to reflect interactions between the design factors. These equations are included in full in Table 4-10 (Model G) and Table 4-11 (Model F). Associated statistics describing how well the models fit the data (PRESS, adequate precision) and their predictive capability (adjusted and predictive R-squared values) are included in Table 4-10 and Table 4-11 to allow the reader to assess robustness and reliability of generated models.

Analysis of panel data for the citrus-like flavour attribute in Model F, resulted in a predictive model for which only level of fructose influenced perception of citrus-like flavour ($p<0.0001$). This was not in agreement with previous studies in the same system (but without carbonation) described in Chapter 3 where both fructose and citric acid were significant design factors.

However, the adjusted and predictive R^2 values were lower for modelling of this attribute than for any other attribute across the two Models (Table 4-11) and the adequate precision (a measure of the signal to noise ratio) for this model was only 7.3 (adequate precision for modelling of other attributes ranges between 16.5 to 49.6). On inspection of the data, the mean score for flavour intensity of one sample was deemed an outlier, falling outside the limits of +/- 2.5 standard deviations. The 'outlier t' measure calculates the number of standard deviations the actual value deviates from the value predicted after deleting the point in question. Analysis of the model with this sample removed returned a predictive model in which factor B (citric acid)

almost reached significance (p=0.0544). In this model the adjusted and predictive R^2 values are higher (0.74 and 0.67 respectively) which would indicate this model better represents the majority of the data set collected for citrus-flavour perception. Both predicted models from analysis of the data with and without the outlier sample are included in Table 4-11.

Table 4-10: Predictive equations (in actual factors) generated for Model G design attributes.

				significant model terms					model statistics				
attribute	CO_2 Level		intercept	glucose	citric acid	glucose^2	citric acid^2	gluc*acid	PRESS	R^2	Adj R^2	Pred R^2	Adeq Precision
overall fizziness	none	sqrt(fizz) =	0.38						0.83	0.96	0.96	0.95	28.72
	low		1.93										
	high		2.73										
tingling	none	sqrt(ting) =	0.28	+ 5x10^{-4}	+ 0.13				0.48	0.99	0.98	0.97	28.31
	low		2.00	- 3.47	+ 0.29								
	high		2.89	- 1.52	- 0.07								
drying in mouth	none	Ln(drying) =	0.21	- 6.5x10-3	+ 0.98				3.30	0.99	0.98	0.95	27.75
	low		1.38	- 4.4x10-3	+ 0.39								
	high		1.87	- 2.5x10-3	+ 0.06								
irritant	none	irritant =	0.48	- 0.014	+ 0.62	+ 1.27x10-4			1.12	0.98	0.96	0.87	27.00
	low		3.64	- 0.028	+ 0.83	+ 1.27x10-4							
	high		6.10	- 0.023	- 0.15	+ 1 27x10-4							
citrus flavour	none	flavour =	3.60	+ 0.018	+ 1.74	- 9.3x10-5			3.21	0.97	0.93	0.85	16.48
	low		3.13	+ 0.026	+ 0.9	- 9.3x10-5							
	high		3 16	+ 0 025	+ 0 96	- 9 3x10-5							
sweetness	none	sweetness =	0.27	+ 0.061	- 0.083		- 7.1x10-3		2.61	0.99	0.99	0.99	45.67
	low		0.37	+ 0.05	- 0.083		- 7.1x10-3						
	high		0 29	+ 0 048	- 0 083		- 7.1x10-3						
sourness	none	Ln (sourness) =	0.24	- 5.5x10-3	+ 1.17				1.39	0.95	0.92	0.82	21.76
	low		1.14	- 5.5x10-3	+ 0.62								
	high		1.51	- 5 5x10-3	+ 0.36								
bitter aftertaste	none	bitter AT =	2.64	- 0.014					11.44	0.81	0.77	0.67	13.56
	low		4.10	- 0.014									
	high		4 86	- 0 014									
acidic aftertaste	none	acidic AT =	1.30	- 6.6x10-3	+ 3.9		- 0.013		10.49	0.98	0.96	0.85	28.95
	low		3.22	- 6.6x10-3	+ 3.0		- 0.013						
	high		4 17	- 6 6x10-3	+ 2 8		- 0 013						
drying aftertaste	none	1/sqrt(drying AT) =	0.78	+ 1.64x10-3	- 0.26				0.02	0.99	0.99	0.96	45.44
	low		0.49	+ 8.2x10-4	- 0.08								
	high		0 41	+ 5 1x10-4	- 0 02								

sqrt – square root, Ln – natural log, 1/sqrt – inverse square root

Table 4-11: Predictive equations (in actual factors) generated for Model F design attributes.

				significant model terms					model statistics				
attribute	CO_2 Level		intercept	fructose	citric acid	fructose^2	citric acid^2	fruct*acid	PRESS	R^2	Adj R^2	Pred R^2	Adeq Precision
overall fizziness	none	sqrt(fizz) =	0.39		- 0.29		+ 0.22		0.18	0.99	0.99	0.99	49.63
	low		1.88		- 0.29		+ 0.22						
	high		2.55		- 0.29		+ 0.22						
tingling	none	sqrt(ting) =	0.39		- 0.27		+ 0.24		0.23	0.99	0.99	0.98	44.62
	low		1.99		- 0.27		+ 0.24						
	high		2.60		- 0 27		+ 0.24						
drying in mouth	none	sqrt(drying) =	1.16	- 5.2x10-3	+ 0.74				3.47	0.98	0.97	0.95	27.35
	low		2.21	- 5.2x10-3	+ 0.19								
	high		2 50	- 5.2x10-3	+ 0.17								
irritant	none	irritant =	1.01		- 1.24		+ 0.94		0.21	0.99	0.98	0.96	35.37
	low		4.14		- 1.24		+ 0.94						
	high		5 82		- 1.24		+ 0.94						
citrus flavour		flavour =	4.61	+ 0.023					7.77	0.56	0.53	0.42	7.34
citrus flavour (outlier removed)		flavour =	4.05	+ 0.030	+ 0.39				4.29	0.78	0.74	0.67	11.99
sweetness	none	sweetness =	0.14	+ 0.12	+ 0.077		- 0.017		4.32	0.99	0.99	0.97	34.69
	low		0.65	+ 0.097	+ 0 077		- 0.017						
	high		0 53	+0 097	+ 0 077		- 0 017						
sourness	none	sourness =	1.28	- 7.1x10-3	+ 3.2		- 0.023		3.32	0.99	0.98	0.94	33.04
	low		3.33	- 0.014	+ 2.10		- 0.023						
	high		3 89	- 0 026	+ 2 75		- 0 023						
bitter aftertaste	none	bitter AT =	2.86	- 0.034	+ 0.42		+ 9.6x10-3		11.19	0.98	0.95	0.72	20.68
	low		6.22	- 0.06	- 0.27		+ 9.6x10-3						
	high		6.13	- 0.041	- 0 85		+ 9 6x10-3						
acidic aftertaste	none	acidic AT =	2.06	- 0.019	+ 2.10				5.80	0.95	0.93	0.90	24.70
	low		3.39	- 0.019	+ 2.10								
	high		4.21	- 0 019	+ 2.10								
drying aftertaste	none	drying AT =	2.46	- 0.02	+ 1.86				2.22	0.98	0.97	0.95	31.49
	low		5.30	- 0.02	+ 0.74								
	high		6 04	- 0 02	+ 0 74								

sqrt – square root, Ln – natural log

These tables describe the predictive equations in actual values of significant ($p<0.5$) design factors. The influence of carbonation can be identified by a changing numerical value of the intercept and the direction of this change indicates impact of this factor. For example, in Model G (Table 4-10), the perception of 'fizziness' increases with carbonation level as indicated by increasing numerical value of the intercept.

Interactions between design factors signify the influence of one factor on perception is modified dependant on the level of a second factor. These interactions are identified in the predictive equations by the presence of a significant sugar*acid term. In addition, interactions between sugar or acid and carbonation are identified by changing numerical values for the tastant term dependant on CO_2 level. For example, in Model G (Table 4-10) perception of 'drying, in-mouth' was influenced by carbonation (increasing intercept values), and both glucose and citric acid. The effect of both tastants was dependant on the level of carbonation present: increasing carbonation level reduces the weighting of the glucose or citric acid concentration on the predictive model.

The equations generated can be used to predict perception of an attribute for any concentration of design factors (acid, sugar, carbonation) within the model design space. For example, in Model G (Table 4-10), sweetness perception of a beverage containing 100g/l glucose, 1g/l citric acid and the low level of carbonation can be calculated thus:

$$\text{'sweetness'} = 0.37 + G*(0.05) - CA*(0.083) - GCA*(7.1\times10^{-3})$$

where G=amount of glucose, CA= amount of citric acid

Therefore,

$$\text{'sweetness'} = 0.37 + 100*(0.05) - 1*(0.083) - (100*1)*(7.1\times10^{-3})$$

$$\text{'sweetness'} = 4.58$$

So, for a beverage of the described composition, the model predicts a perception of sweetness of 4.58 when assessed in the same manner as previously described (section 4.2.4.3). These predictive ratings can be used to validate the generated models (see section 4.3.6)

As a result of the number of variables included in the design spaces for Models G and F, these tables describing the predictive models are somewhat unwieldy. For ease of review, summary tables detailing design terms included in predictive equations for each attribute and model are shown in Table 4-12 and Table 4-13. This enables the reader to rapidly identify design factors and interaction terms which significantly influenced perception of each attribute. Furthermore, 2 statistical measures, relating model fit of the data (adjusted R^2) and predictive capacity of the model (predicitve R^2) are also included in Table 4-12 and Table 4-13.

Table 4-12: Design factors involved in predictive models generated for Model G (glucose/citric acid/carbonation).

Attribute	Terms in Predictive Model	Adj R_2	Pred R_2
fizz	CO_2	0.96	0.95
tingling	G, ca, CO_2, $GC0_2$, $CACO_2$	0.98	0.97
drying	G, CA, CO_2, GCO_2, $CACO_2$	0.96	0.87
irritating	g, CA, CO_2, G^2, GCO_2, $CACO_2$	0.98	0.95
flavour	G, CA, co_2, G^2, GCO_2, $CACO_2$	0.93	0.85
sweetness	G, CA, CO_2, GCA, GCO_2	0.99	0.99
sourness	G, CA, CO_2, $CACO_2$	0.92	0.82
bitter AT	G, CO_2	0.77	0.67
acidic AT	G, CA, CO_2, GCA, $CACO_2$	0.96	0.85
drying AT	G, CA, CO_2, GCA, $CACO_2$	0.99	0.96

AT- aftertaste, G- glucose, CA- citric acid,
Terms in lowercase are not significant factors in model but are required for model hierarchy.

Table 4-13: Design factors involved in predictive models generated for Model F (fructose/citric acid/carbonation).

Attribute	Terms in Predictive Model	Adj R_2	Pred R_2
fizz	ca, CO_2, CA^2	0.99	0.99
tingling	CA, CO_2, CA^2	0.99	0.98
drying	F, CA, CO_2, $CACO_2$	0.98	0.96
irritating	ca, CO_2, CA^2	0.97	0.95
flavour	F, CA	0.74	0.67
sweetness	F, CA, co_2, FCA, FCO_2	0.99	0.97
sourness	F, CA, CO_2, FCA, FCO_2, $CACO_2$	0.98	0.94
bitter AT	F, CA, CO_2, fca, FCO_2, $CACO_2$	0.95	0.72
acidic AT	F, CA, CO_2	0.93	0.9
drying AT	F, CA, CO_2, $CACO_2$	0.97	0.95

AT- aftertaste, F- fructose, CA- citric acid,
Terms in lowercase are not significant factors in model but are required for model hierarchy

Contour and interaction plots generated by the predictive models can be used to visualise the data to identify effect of changing concentration of design factors. These plots are used to examine each attribute in greater detail and allow the impact of predictive model terms on perception of attributes to be clarified in the following sections.

4.3.5.1. Overall Fizziness

- '*overall perception in the whole mouth including both bubbling feeling and pain perception*'

Analysis of mean panel data for the attribute 'overall fizziness' (Table 4-8: Model G, Table 4-9: Model F) showed a significant difference between samples. Examination of multiple comparison test groupings together with sample composition data (Table 4-14) clearly indicated 'fizziness' was highly correlated with carbonation level.

Table 4-14: Analysis of attribute 'fizziness'.

a

Sample/ product	glucose (g/l)	citric acid (g/l)	CO_2 Level	Panel Mean	Groups
2	0	0	high	8.03	A
11	0	1.5	high	7.96	A
14	37.5	0.75	high	7.52	A
13	150	0	high	7.24	A
9	150	0	high	7.21	A
3	150	1.5	high	6.67	AB
10	0	0.75	low	5.30	BC
15	75	1.5	low	5.00	C
16	150	0.75	low	3.83	CD
6	75	1.5	low	3.78	CD
17	0	0	low	3.65	CD
7	150	0.75	low	2.77	D
5	75	0	low	2.17	D
1	150	1.5	none	0.24	E
12	75	0.75	none	0.24	E
4	0	1.5	none	0.13	E
18	150	0	none	0.09	E
8	0	0	none	0.07	E

b

Sample/ product	fructose (g/l)	citric acid (g/l)	CO_2 Level	Panel Mean	Groups
18	0	1.5	high	7.02	A
6	64	0	high	6.67	A
2	64	1.5	high	6.47	A
11	0	0 75	low	6.23	A
14	32	0.75	high	6.23	A
1	0	0	high	6.14	A
7	32	0	low	4.13	B
9	32	1 5	low	3 86	B
16	32	1.5	low	3.68	B
13	0	0	low	3.44	B
4	64	0 75	low	3.22	B
3	64	0.75	low	2 9	B
12	64	1 5	none	0.29	C
10	64	0	none	0.16	C
5	16	0 75	none	0.11	C
8	0	0	none	0.1	C
15	0	1 5	none	0.1	C
17	64	0	none	0.09	C

Where a=Model G, b=Model F

This relationship was reflected in the predictive models for this attribute (Table 4-12 and Table 4-13) and can be clearly seen in the interaction plots (Figure 4-6). Data for Model G indicates only carbonation level was a significant factor in this perception of 'overall fizziness' (C; $p<0.0001$). Model F mimics this (C; $p<0.0001$), but also has terms relating to the citric acid concentration such that higher levels of acid appear to cause slight enhancement of perception of fizziness. However, the term for citric acid alone (B) is not significant ($p=0.20$), but is needed to retain model hierarchy for inclusion of the term B^2 which does significantly impact on the attribute ($p=0.027$) although this has much lower weighting in the predictive equations (Table 4-11) when compared to impact of CO_2 in low and high carbonated samples.

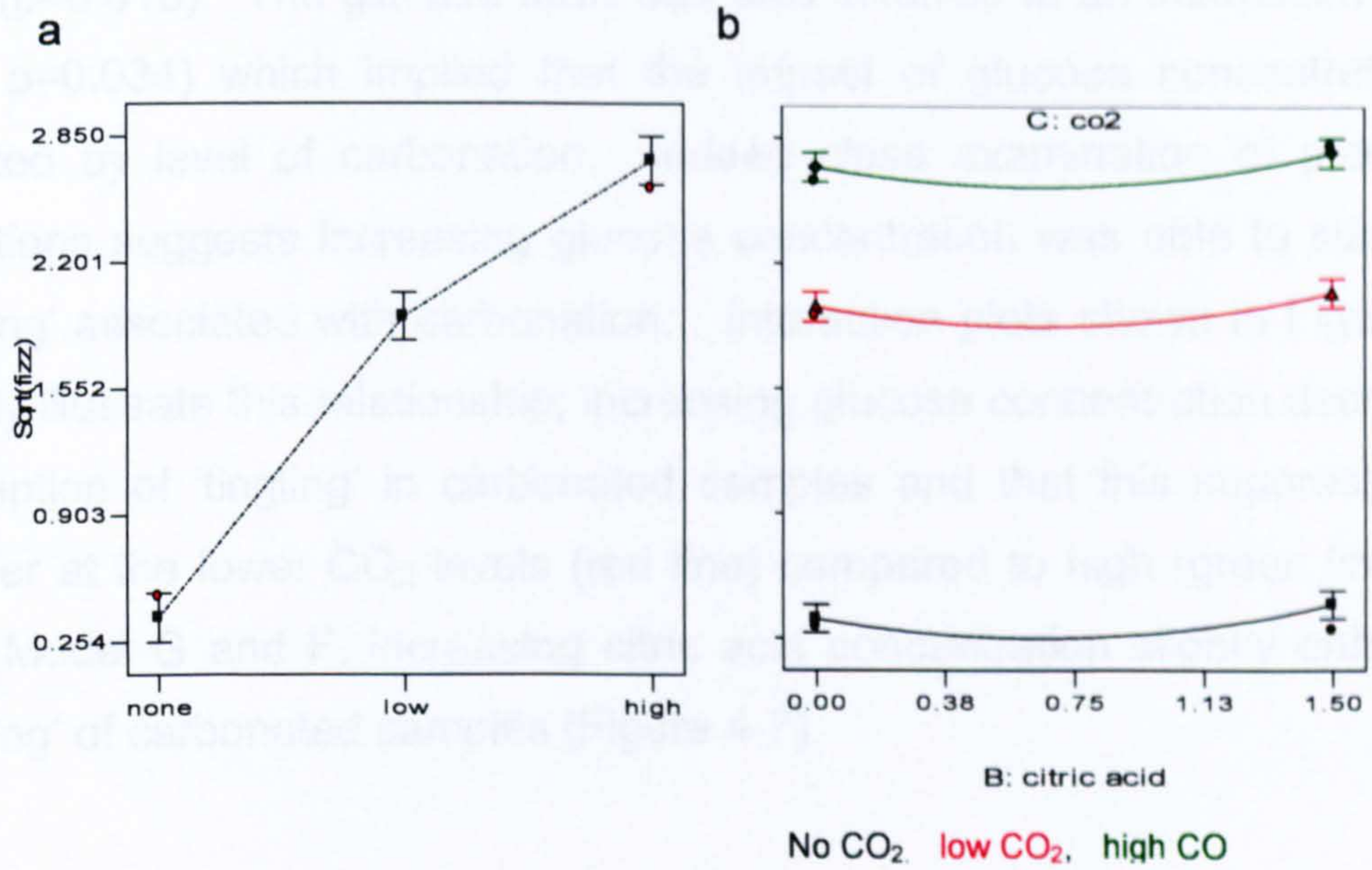


Figure 4-6: Interaction plots describing 'overall fizziness' attribute (a-Model G, b-Model F).

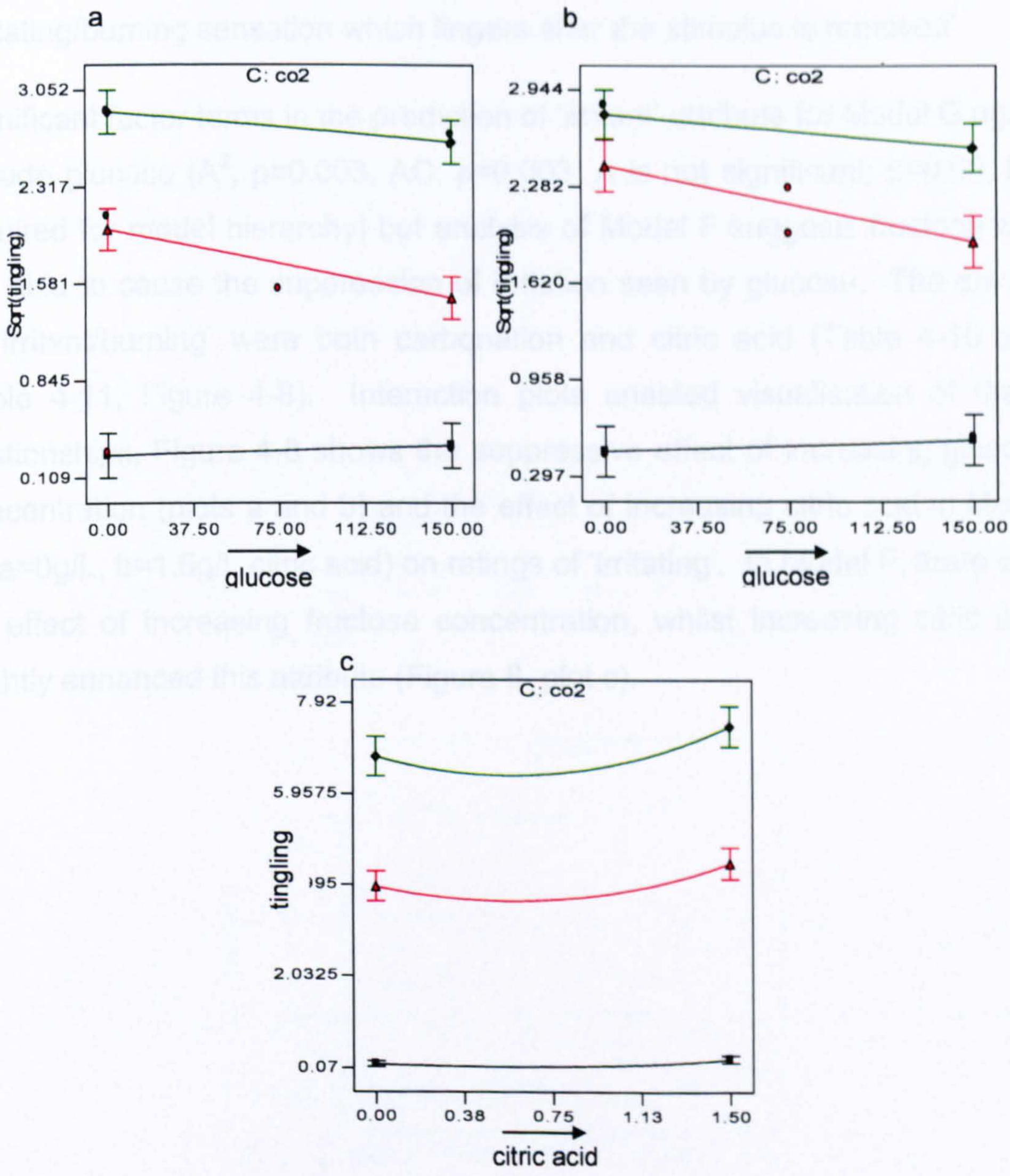
4.3.5.2. *Tingling*

- *'sensation associated with fizz/acidity on tongue and around inside of mouth – like a pins and needles sensation'*

ANOVA analysis of panel data showed significant differences in rating of 'tingling' existed between the samples in both Model designs. However, inspection of multiple comparison test data did not clearly illustrate how design factors were influencing perception of this attribute. Therefore interaction plots generated by the predictive models were used to visualise the data and identify effects of changing concentration of design factors on perceptual scores (Figure 4-7).

The predictive modelling and resultant equations show that, for Model F, the same factors involved in 'overall fizziness' are significant for this attribute also (Table 4-9, B; $p=0.05$, B^2; $p=0.04$). However, analysis of Model G, indicates that additionally, glucose concentration was important in predicting

perception of this attribute, indicated by inclusion of glucose as a significant term (p=0.015). The glucose term was also involved in an interaction factor (AC, p=0.034) which implied that the impact of glucose concentration is affected by level of carbonation. Indeed close examination of predictive equations suggests increasing glucose concentration was able to suppress 'tingling' associated with carbonation. Interaction plots shown in Figure 4-7 clearly illustrate this relationship; increasing glucose concentration decreases perception of 'tingling' in carbonated samples and that this suppression is greater at the lower CO_2 levels (red line) compared to high (green line). In both Model G and F, increasing citric acid concentration slightly enhanced 'tingling' of carbonated samples (Figure 4-7).



CO_2= none, CO_2= low, CO_2= high

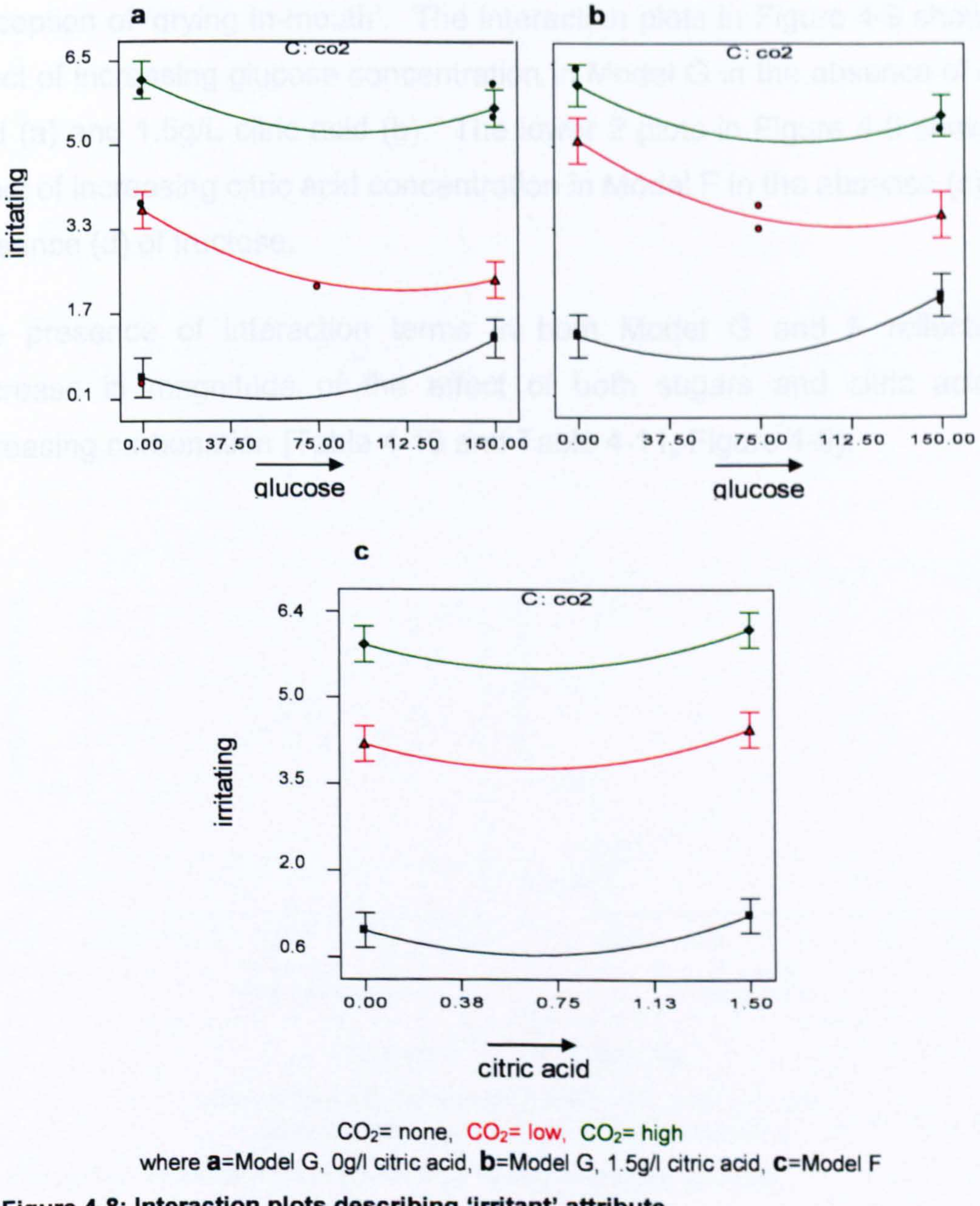
where **a**=Model G, 0g/l citric acid, **b**=Model G, 1.5g/l citric acid, **c**=Model F

Figure 4-7: Interaction plots describing 'tingling' attribute

4.3.5.3. *Irritant/burning*

'Irritating/burning sensation which lingers after the stimulus is removed'

Significant factor terms in the prediction of 'irritant' attribute for Model G again include glucose (A^2; p=0.003, AC; p=0.003, A is not significant; p=0.09, but required for model hierarchy) but analysis of Model F suggests fructose was not able to cause the suppression of irritation seen by glucose. The drivers of 'irritant/burning' were both carbonation and citric acid (Table 4-10 and Table 4-11, Figure 4-8). Interaction plots enabled visualisation of these relationships, Figure 4-8 shows the suppressive effect of increasing glucose concentration (plots a and b) and the effect of increasing citric acid in Model G (a=0g/L, b=1.5g/L citric acid) on ratings of 'irritating'. In Model F, there was no effect of increasing fructose concentration, whilst increasing citric acid slightly enhanced this attribute (Figure 8, plot c).



CO_2= none, CO_2= low, CO_2= high

where **a**=Model G, 0g/l citric acid, **b**=Model G, 1.5g/l citric acid, **c**=Model F

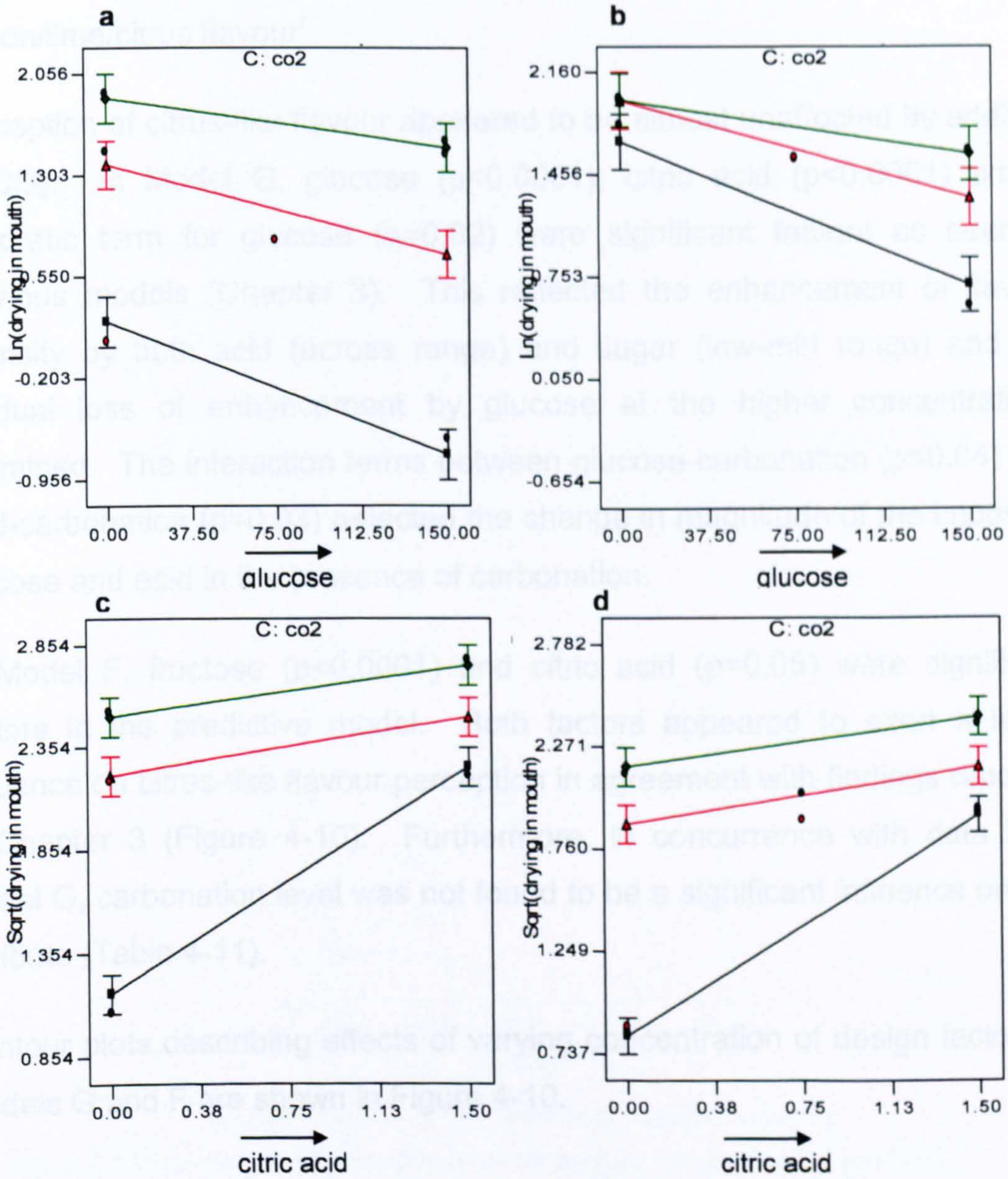
Figure 4-8: Interaction plots describing 'irritant' attribute

4.3.5.4. Drying in-mouth

'tactile sensation due to shrinking, drawing or puckering of oral epithelium'

Predictive equations for 'drying in-mouth' showed that all 3 design factors influenced this attribute in both Models G and F (A; $p<0.0001$, B; $p<0.0001$, C; $p<0.001$). Increasing either sugar resulted in suppression whilst increasing citric acid and/or carbonation resulted in enhancement of the perception of 'drying in-mouth'. The interaction plots in Figure 4-9 show the effect of increasing glucose concentration in Model G in the absence of citric acid (a) and 1.5g/L citric acid (b). The lower 2 plots in Figure 4-9 show the effect of increasing citric acid concentration in Model F in the absence (c) and presence (d) of fructose.

The presence of interaction terms in both Model G and F reflected a decrease in magnitude of the effect of both sugars and citric acid on increasing carbonation (Table 4-10 and Table 4-11, Figure 4-9).



CO_2= none, CO_2= low, CO_2= high

where **a**=Model G, 0g/l citric acid, **b**=Model G, 1.5g/l citric acid,
c=Model F, 0g/l fructose, **d**=Model F, 64g/l fructose

Figure 4-9: Interaction plots describing 'drying in-mouth' attribute

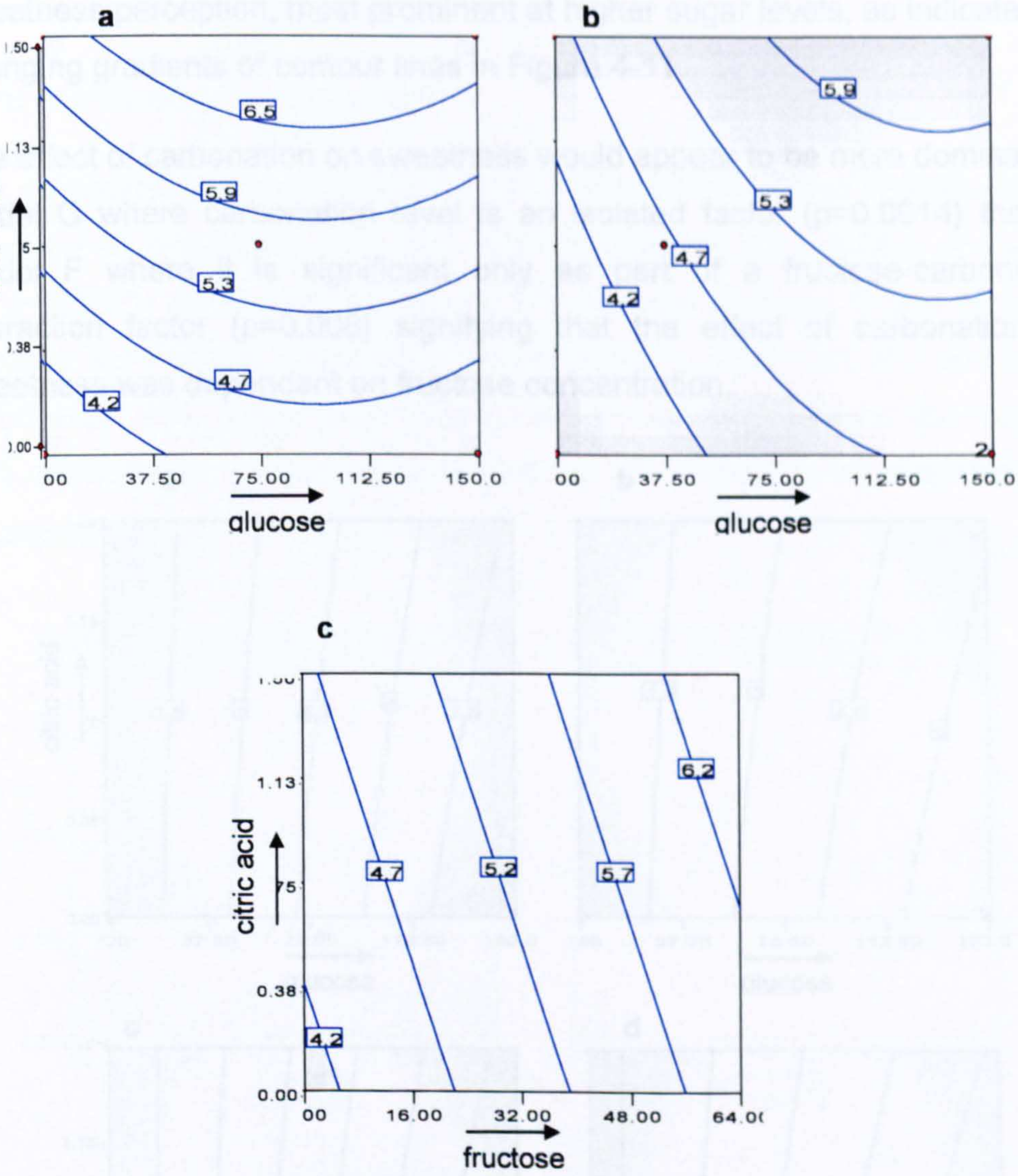
4.3.5.5. *Citrus-like flavour*

'lemon/lime/citrus flavour'

Perception of citrus-like flavour appeared to be almost unaffected by addition of CO_2. In Model G, glucose ($p<0.0001$), citric acid ($p<0.0001$) and a quadratic term for glucose ($p=0.02$) were significant factors as seen in previous models (Chapter 3). This reflected the enhancement of flavour intensity by both acid (across range) and sugar (low-mid range) and the gradual loss of enhancement by glucose at the higher concentrations examined. The interaction terms between glucose-carbonation ($p=0.04$) and acid-carbonation ($p=0.03$) reflected the change in magnitude of the impact of glucose and acid in the presence of carbonation.

In Model F, fructose ($p<0.0001$) and citric acid ($p=0.05$) were significant factors in the predictive model. Both factors appeared to exert a linear influence on citrus-like flavour perception in agreement with findings reported in Chapter 3 (Figure 4-10). Furthermore, in concurrence with data from Model G, carbonation level was not found to be a significant influence on this attribute (Table 4-11).

Contour plots describing effects of varying concentration of design factors in Models G and F are shown in Figure 4-10.



where; **a**=Model G, no CO_2, **b**=Model G, high CO_2, **c**=Model F

Figure 4-10: Contour and interaction plots describing 'citrus- flavour' attribute

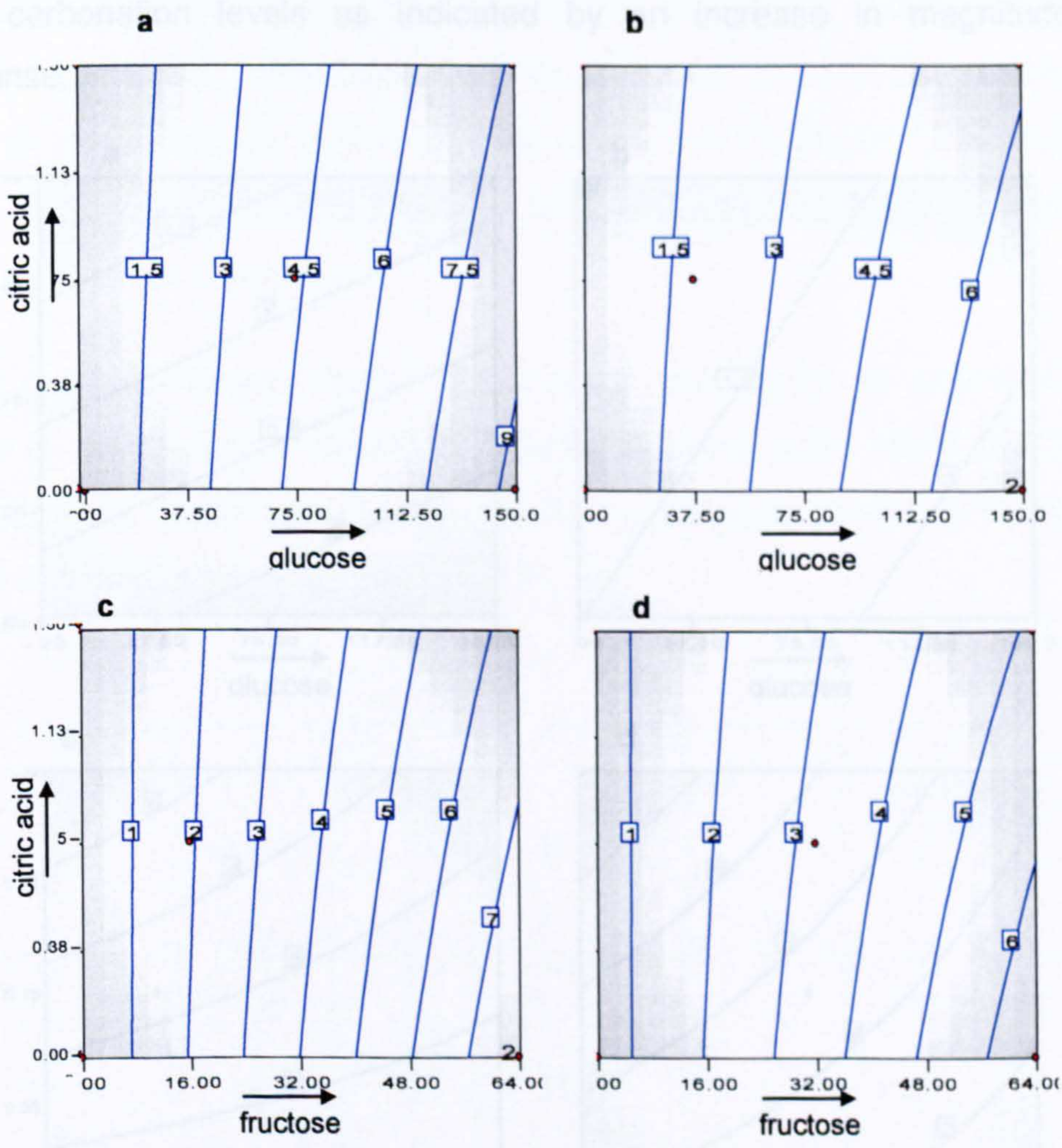
4.3.5.6. Sweetness

'taste stimulated by sugar in water'

Analysis of panel data of sweetness scores demonstrated that as expected, increasing levels of both sugars resulted in an increase in perception of sweetness. The modelling of this attribute also revealed addition of both carbonation and increasing levels of citric acid caused a suppression of

sweetness perception, most prominent at higher sugar levels, as indicated by changing gradients of contour lines in Figure 4-11.

The effect of carbonation on sweetness would appear to be more dominant in Model G where carbonation level is an isolated factor (p=0.0014) than in Model F where it is significant only as part of a fructose-carbonation interaction factor (p=0.008) signifying that the effect of carbonation on sweetness was dependant on fructose concentration.



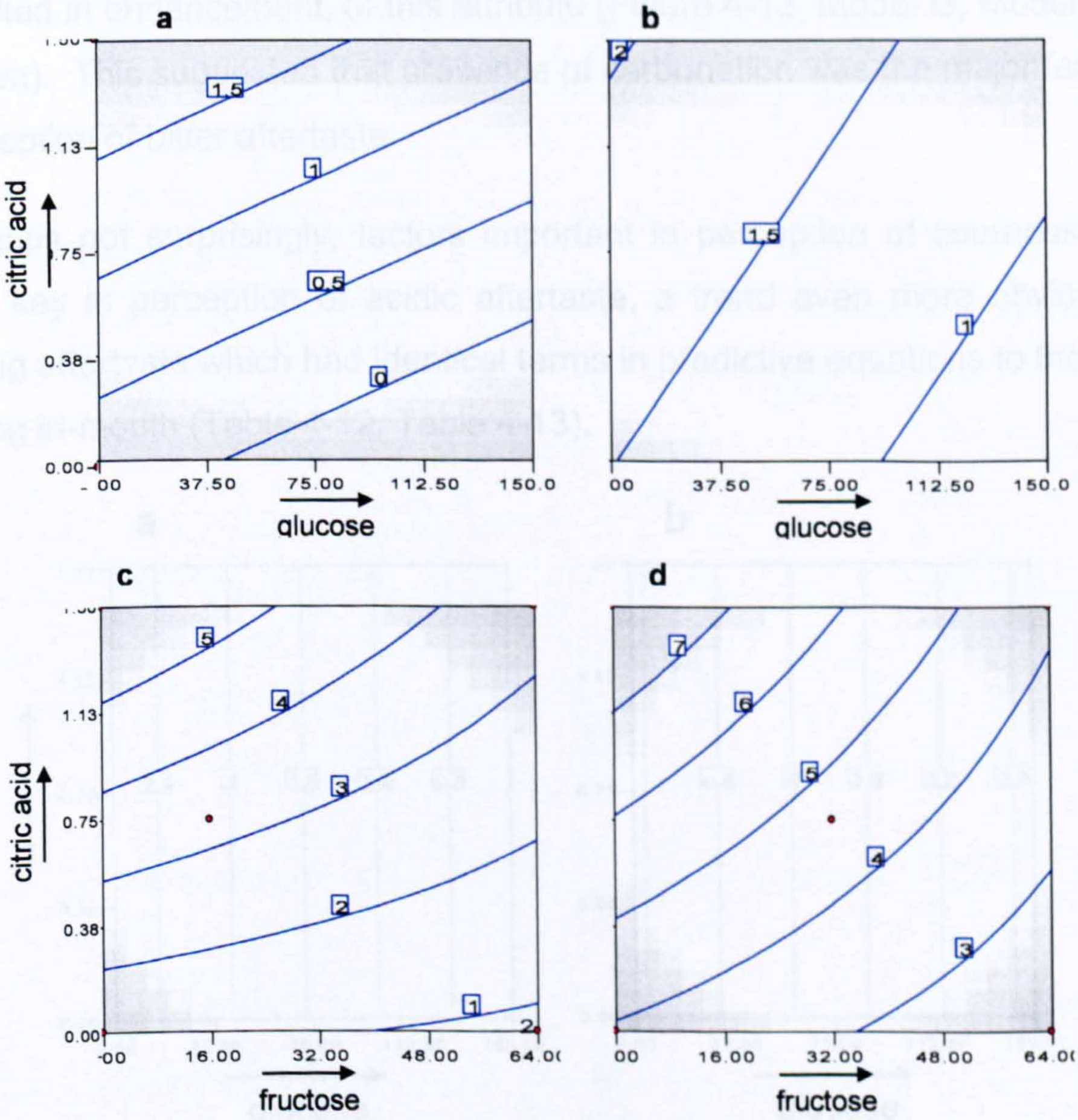
where; **a**=Model G, no CO_2, **b**=Model G, high CO_2, **c**=Model F, no CO_2, **d**=Model F, high CO_2

Figure 4-11: Contour plots describing 'sweetness' attribute

4.3.5.7. *Sourness*

'taste stimulated by acid in water'

The perception of sourness was driven primarily by the level of citric acid as expected (B; $p<0.0001$). However, both carbonation (C; $p<0.001$) and sugar (A; $p<0.0001$) significantly influenced this attribute in Model G and Model F. Glucose and fructose suppressed the perception of sourness, and addition of carbonation enhanced it, with an effect being seen even between the low and high carbonation levels as indicated by an increase in magnitude of response.



where; **a**=Model G, no CO_2, **b**=Model G, high CO_2, **c**=Model F, no CO_2, **d**=Model F, high CO_2

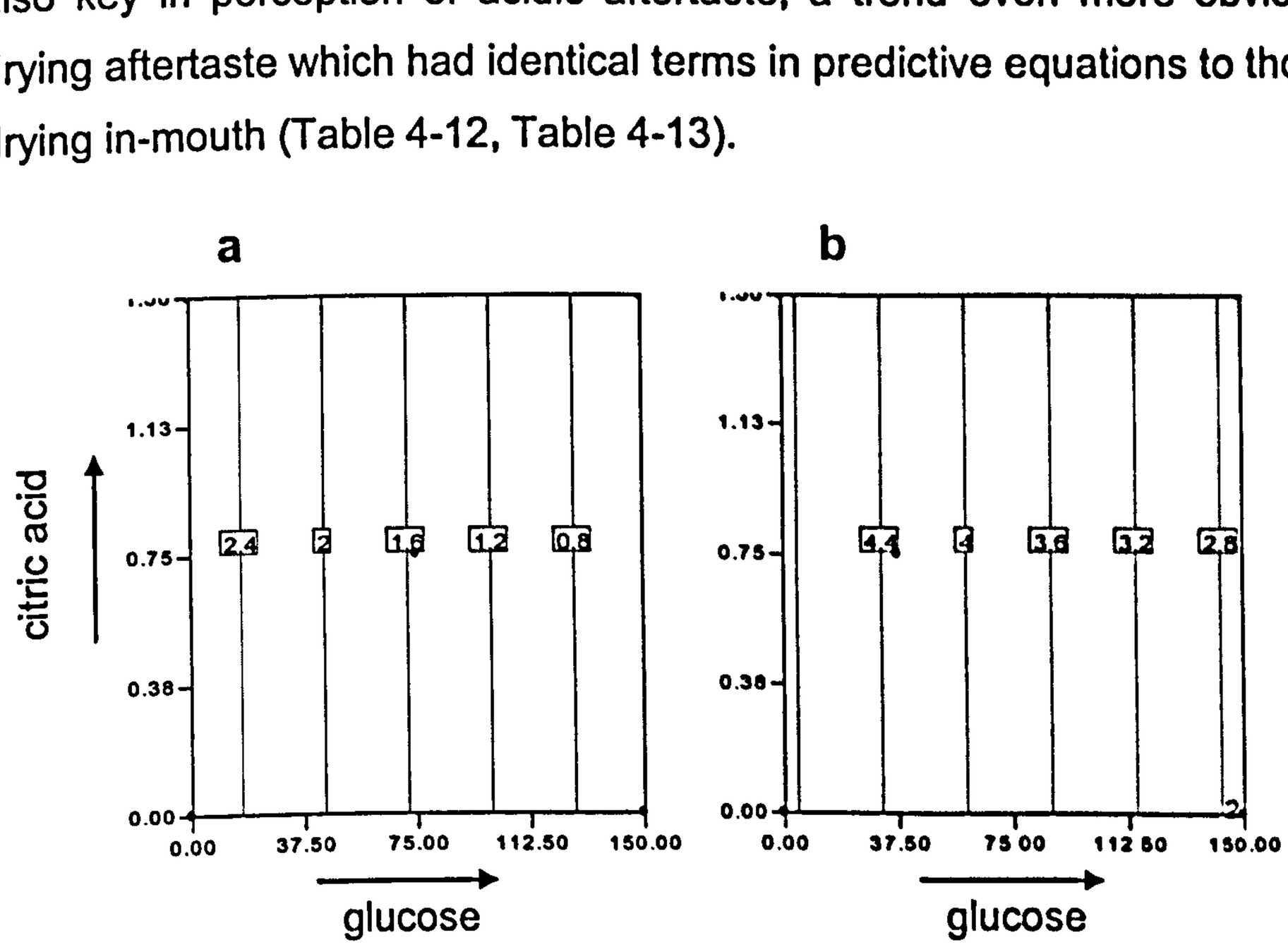
Figure 4-12: Contour plots describing 'sourness' attribute

4.3.5.8. *Aftertastes; bitter, acidic, drying*

'aftertaste stimulated by caffeine in water', 'aftertaste stimulated by acid in water', 'aftertaste tactile sensation due to shrinking, drawing or puckering of oral epithelium'

Both Models G and F (Table 4-12 and Table 4-13) showed sugar and carbonation levels were significant factors impacting on the predictive modelling of the bitter aftertaste attribute. Increasing either glucose or fructose suppressed, and addition and increase in level of carbonation resulted in enhancement, of this attribute (Figure 4-13, Model G, Model F not shown). This suggested that presence of carbonation was the major factor in perception of bitter aftertaste.

Perhaps not surprisingly, factors important in perception of sourness were also key in perception of acidic aftertaste, a trend even more obvious for drying aftertaste which had identical terms in predictive equations to those for drying in-mouth (Table 4-12, Table 4-13).



Model G where **a**= no CO_2 and **b**=high CO_2

Figure 4-13: Contour plots describing 'bitter aftertaste' attribute

4.3.6. Model Validation

Experimentally obtained scores for attribute intensities for each sample were plotted against the scores predicted by the models. Good correlation was observed between the predicted and actual scores for the data from the set of samples used for Model G and Model F. In addition, the attribute ratings of the independent set of validation samples showed adequate agreement between actual and model-predicted scores. Examples of plots detailing the relationship between actual versus model-predicted scores are given for attributes 'tingling' (a), 'citrus-like flavour' (b) and 'bitter aftertaste' (c) from Model G.

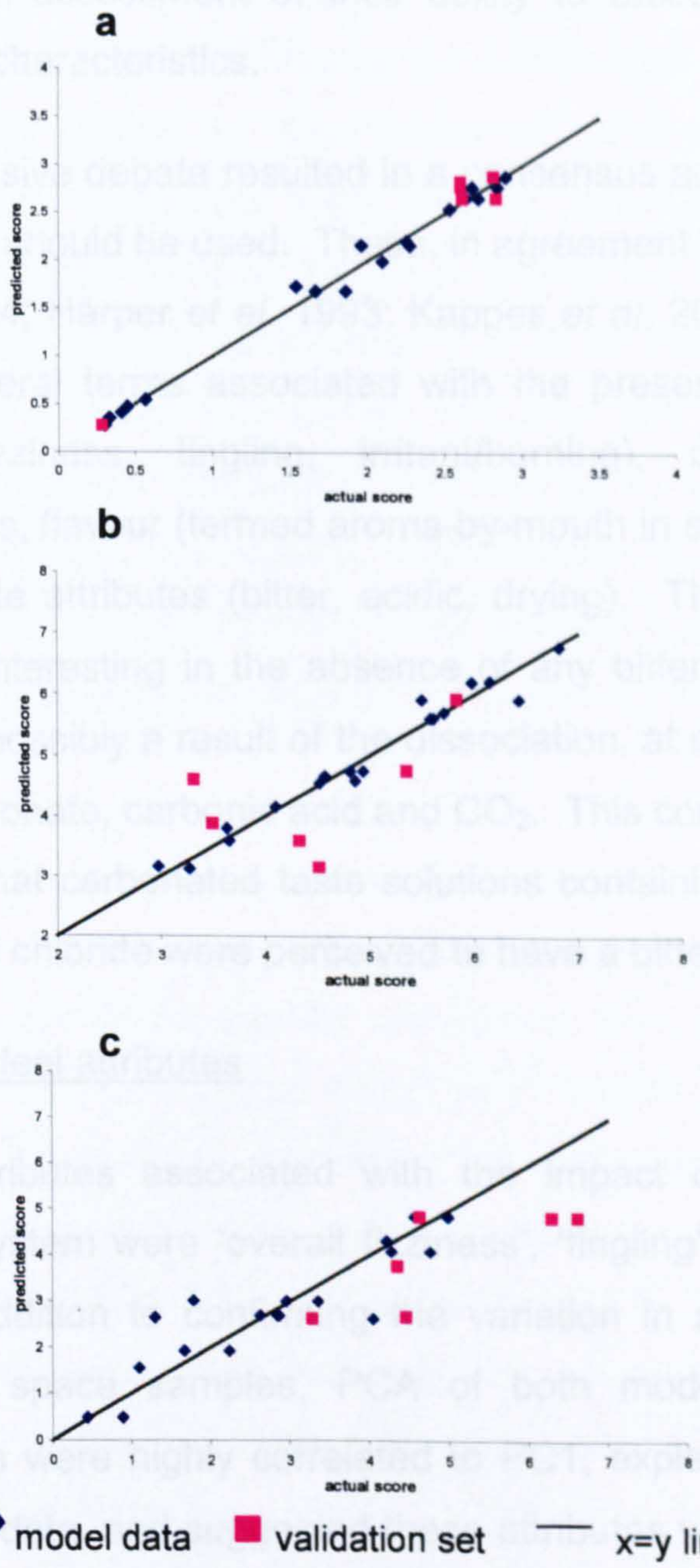


Figure 4-14: Predicted versus actual scores for 'tingling' (a), 'citrus flavour' (b) and 'bitter aftertaste' (c) of both model samples and validation set for Model G.

4.4. Discussion

The use of a trained panel of assessors and their development of a lexicon of attributes to describe and discriminate between the samples used in these studies was crucial. The assessors had previously undergone screening

tests to establish sensory acuity to basic taste solutions and a range of odorants as well as assessment of their ability to articulate verbally and notionally sensorial characteristics.

Thorough and extensive debate resulted in a consensus across the panel as to which descriptors should be used. These, in agreement with other studies, (McLellan *et al.* 1984; Harper *et al.* 1993; Kappes *et al.* 2006; Kappes *et al.* 2006) included several terms associated with the presence of bubbles in mouth (overall fizziness, tingling, irritant/burning), drying/astringency, sweetness, sourness, flavour (termed aroma-by-mouth in studies by Kappes (2006) and aftertaste attributes (bitter, acidic, drying). The perception of a bitter aftertaste is interesting in the absence of any bitter compound within the designs and is possibly a result of the dissociation, at equilibrium, of CO_2 to carbonate, bicarbonate, carbonic acid and CO_2. This corroborates findings by Cowart (1998) that carbonated taste solutions containing solely sucrose, citric acid or sodium chloride were perceived to have a bitter taste quality.

4.4.1. Mouthfeel attributes

The generated attributes associated with the impact of carbonation on mouthfeel in this system were 'overall fizziness', 'tingling', 'drying in-mouth' and 'irritant'. In addition to confirming the variation in sensory properties within the design space samples, PCA of both models indicated that mouthfeel attributes were highly correlated to PC1, explaining ~45% of the variation within the data, and suggested these attributes were a main source of discrimination between the samples.

The first of these; 'overall fizziness' was driven by the carbonation level and to a minor degree in Model F, by citric acid concentration, which at the high end of the concentration range examined appeared to enhance the perception of fizziness to a small degree (Figure 4-6).

In a similar way, carbonation level was also the major driver for 'tingling' and 'irritant' perception but in these instance glucose concentration exerted an

influence but fructose level did not (Figure 4-7 and Figure 4-8). By increasing the amount of glucose, the sensation of 'tingling' and 'irritant' was suppressed and this effect was greater at low carbonation level compared to high. This may be linked to the choice of perceptually equi-sweet concentrations of sugars within the study designs. As fructose has a higher sweetness index than glucose this resulted in double the amount of glucose compared to fructose and this factor may impact on the mouthfeel of the samples generated; although equi-sweet, viscosity may be altered, which could impact on perceptual attributes. Instrumental measures of viscosity (Chapter 2) showed only the highest concentration of glucose (150g/L) differed significantly in viscosity from the other samples and a recent study by Kappes *et al* (2007) suggests the small increase seen (0.4mPa.s) may not be identifiable 'in-mouth'.

Citric acid was significant factor in the predictive models generated for both designs and appears to enhance 'tingling' but this effect is again minimal in Model F and only influential at the low carbonation level in Model G. Citric acid appears to cause a small initial reduction in 'irritant' intensity, an effect which is confined primarily to low and high carbonation levels. This suppression decreases in magnitude until followed by eventual enhancement at higher acid levels (Figure 4-8). This enhancement of 'irritant' intensity by citric acid agrees with previous studies suggesting high levels of acids elicit an irritant sensation in addition to sour taste (Gilmore *et al.* 1993; Dessirier *et al.* 2000; Prescott *et al.* 2004).

From current knowledge regarding the mode of action of CO_2, it could be theorised that the both the 'tingling' and 'irritant' sensations may well be related to the action of CO_2 on oral nociceptors, while 'overall fizziness' relates to the sensation of bubbles bursting activating oral mechanoreceptors. The attributes of 'tingling' (defined as 'a pins and needles' feeling) and 'irritant' (defined as 'irritating sensation which lingers after the stimulus is removed'), although clearly defined and separate

perceptual qualities, can be more closely linked to descriptors in previous studies examining the trigeminal action of CO_2 (Dessirier *et al.* 2000; Carstens *et al.* 2002).

Whereas carbonation level was the main/only factor impacting on fizziness as would be expected, the data indicated glucose was able to suppress the trigeminal related response in some way. It is perceivable that the addition of glucose, particularly at high concentration, would change the viscosity of the solution, possibly influencing the formation or size of CO_2 bubbles; perhaps creating a larger number of smaller sized bubbles (Lynch *et al.* 2002; Liger-Belair 2005). As all samples were carbonated after all design components had been added and mixed, it can be assumed that final carbonation levels were not altered by amount of solutes in the sample. This may result in the parity seen in feelings of bubbles bursting within the oral cavity (fizziness) between samples with differing quantities of sugars. Changes in viscosity and/or mouthfeel, resulting from addition of high levels of glucose, may 'coat' the tongue, 'softening' the impact.

Alternatively, the glucose molecules may modify the production of carbonic acid, perhaps by obstructing CO_2 coming into contact with carbonic anhydrase, a requisite for its conversion to carbonic acid. Although it is not clear where this conversion occurs, Dessirier *et al* (2000) offer two suggestions; an extracellular site which would allow a common method of nociceptor excitation by both CO_2 and acids involving acid-sensitive ion channels, or an intracellular site where CO_2 is converted to carbonic acid within the nociceptor fibre terminal. Additional evidence from electrophysiological studies (Komai *et al.* 1993) showing some lingual nerve fibres respond only to CO_2 and not merely acidic solutions would favour the second option.

Decoupling the sensations due to mechanical and chemogenic actions of CO_2, would require blocking of one or the other; by either carbonic anhydrase inhibitors or use of a hyperbaric chamber preventing the formation of

bubbles. As previously mentioned (4.1.1), studies using carbonic anhydrase inhibitors (Simons *et al.* 1999; Dessirier *et al.* 2000) showed a decrease in reported intensity of the sensation of carbonation (but not total ablation) and McEvoy (1998) reported consumption of carbonated water under hyperbaric conditions, preventing bubble formation, still elicited sensations described as 'tingling', 'mouth burn' and 'pricking'. Whilst tempting to conclude glucose may impact on the chemogenic, rather than mechanical, action of CO_2, full investigations disconnecting the dual actions of carbonation should be undertaken.

4.4.2. Taste and flavour attributes

The effect of carbonation on perception of citrus-flavour in these systems is varied. In Model G, presence of carbonation suppresses perceived flavour intensity slightly but also reduces the enhancement of flavour by citric acid as seen by the changing gradient of contour lines in Figure 4-10. The contour plots describing the predictive model generated for Model G show the same plateau in the enhancement of flavour intensity by glucose at higher levels as seen in the previous study (Chapter 3) whilst the enhancement caused by citric acid is again linear.

The predictive model generated by the data from Model F suggests (both fructose and citric acid enhanced flavour perception (Figure 4-10), in agreement with the findings in the previous chapter (Chapter 3). Nevertheless, the data presents no evidence to support an influence of carbonation on citrus flavour perception in this model design.

Both Models G and F show an impact of carbonation level on perceived sweetness intensity. In Model G, sweetness of glucose was suppressed in a linear manner by increasing carbonation whilst data from Model F suggests sweetness of fructose, although suppressed by presence of carbonation, did not decrease further on increasing carbonation level (Figure 4-11). Whilst not in full agreement with findings by Cometto-Muniz *et al* (1987), Yau and

Daniel (1992) and Prescott *et al* (2004) who reported no substantial change in sweetness ratings in the presence of carbonation, this is likely a result of allowing assessment of all attributes intrinsic to these beverage systems which, as previously discussed (4.1.2) was a limitation in the published studies.

The suppressive effect of citric acid on sweetness appeared to be concentration dependant with the greatest suppression occurring at high sugar concentration in both models.

Carbonation level was a significant factor in sourness perception within both Model G and F (Table 4-12 and Table 4-13 respectively) and was seen to enhance sourness intensity linearly across the range of citric acid. As expected from prior taste-aroma studies (Chapter 3), both sugars caused a suppression of this sourness and this appeared to be greater at low and high carbonation levels as indicated by the increasing gradient of contour lines in plots describing the predictive models (Figure 4-12). This agrees with previous work (McLellan *et al.* 1984; Comettomuniz *et al.* 1987; Yau *et al.* 1992; Prescott *et al.* 2004) reporting increasing sourness intensity with carbonation and probably is due to the presence of carbonic acid as a consequence of dissociation of CO_2 in solution.

4.4.3. Afterftaste attributes

The perception of a bitter aftertaste appeared to be a result of addition of CO_2, ratings of this attribute were of a low magnitude in non-carbonated samples (Figure 4-13) and increasing between low and high carbonation increased the magnitude of response further. Examination of the contour plots indicated some response due to citric acid in Model F in non-carbonated samples but this enhancement moved to a suppressive effect at the high carbonation level. Model G displayed no impact of citric acid on the predictive models (Table 4-12). Sugar level did impact on bitter aftertaste intensity; both Models G and F showed increasing concentration of sugar

suppressed response intensity, as indicated by contour lines of decreasing strength along both glucose and fructose concentration axes in Figure 4-13. Both design models showed a similar pattern of factors influential in perception of acidic and drying aftertaste intensities (Table 4-12 and Table 4-13). Acidic aftertaste was primarily driven by acid level, with carbonation enhancing and sugar suppressing intensity, as would be expected from results for the sourness attribute, and the two attributes appear to be well correlated. A similar correlation can be seen between the two drying attributes; drying in-mouth and drying aftertaste. Predictive models indicated increasing carbonation and citric acid increased intensity of these attributes whilst increasing sugar suppressed intensity (Figure 4-9).

4.5. Conclusions and Summary

The use of profiling as the sensory methodology in this study has eliminated the uncertainty surrounding potential assessor 'dumping' bias associated with the previous study (Chapter 3), rating pre-determined attributes in separate sessions. The use of the profiling technique also extends previous studies in this area investigating impact of CO_2 primarily on sweetness and sourness perception (McLellan *et al.* 1984; Comettomuniz *et al.* 1987; Nahon *et al.* 1996; Odake 2001; Prescott *et al.* 2004) which have consistently enforced the nature and number of attributes under assessment. This approach would again, leave open the possibility of dumping bias affecting ratings and does not allow for expression of changes in taste quality which may occur within the design space. Using a stimulus such as carbonation, which is known to elicit sensations by way of both oral mechanoreceptors and nociceptors, but may also produce taste sensations in solution at equilibrium. Such a complex system requires appraisal of all allied attributes to fully understand its influence on sensory perception.

A number of interesting and novel results have been generated by the current approach, which also provides additional support for findings,

reported in the previous chapter, regarding the differential effects of the two sugars under investigation. The same pattern of enhancement of citrus flavour perception by glucose and fructose was observed in this study as in the previous despite a change in methodology. In the current method of sensory profiling, ratings of all attributes were allowed whereas preceding results were a consequence of limited scoring options. The finding that acid and sugar interactions with aroma occur, and influence citrus flavour perception in a similar manner in both approaches, lends support to the hypothesis that these are 'real' interactions and not simply a result of 'dumping' bias or taste-smell confusion as previously discussed (Chapter 3).

The results of the current investigation of carbonated systems, support published literature showing addition of CO_2 causes enhancement of sourness and confirmed previously inconclusive evidence of suppression of sweetness by CO_2. Furthermore, data also highlighted differing effects of addition of glucose or fructose on some of the mouthfeel attributes generated to describe carbonation sensations. In particular, 'tingling' and 'irritating' intensity was suppressed by glucose but not by fructose. As the oral sensations associated with carbonated beverages are a major hedonic factor for consumers, it would be of interest to further this finding and assess liking in relation to the 'tingling' attribute; is a more intense sensation preferred or is there a market for a 'gentler', less severe oral experience?

5. Influence of caffeine

5.1. *Introduction*

Sugars and acids are common ingredients in commercial beverages and effects of these tastants on perception have previously been explored in a model beverage system (Chapter 3). However, in addition to compounds possessing a sweet or sour taste quality, bitter taste compounds are also frequently found in commercial drinks. These compounds include caffeine, quinine, artificial sweeteners (e.g saccharin), and preservatives (e.g. sodium benzoate). Furthermore, a consequence of the growing demand for 'functional' beverages is the inclusion of minerals, vitamins and herbal extracts into beverages, many of which have bitter taste notes.

In humans, compounds eliciting a bitter taste response activate a family of 25-30 G-protein coupled receptors (GPCR) termed Tas2Rs (Chandrashekar *et al.* 2000). Current research suggests the predominant G protein composition in bitter taste cells is a combination of α-gustducin, Gβ3 and Gγ13 (Behrens *et al.* 2006).

5.1.1. Individual variation in bitter sensitivity

Individual variation in the human perception of bitter taste compounds has been determined to have a genetic basis. In 1932, Fox discovered sensitivity to a bitter taste compound, phenyl thiocarbamide (PTC), varied between individuals (Fox 1932). PTC was reported to be virtually tasteless to some individuals, whilst perceived as very intensely bitter to others. Research has since focussed on an alternative bitter compound 6-*n*-propylthiouracil (PROP) due to the toxicity of PTC. Individuals can be grouped according to PROP sensitivity into three categories: non-tasters, tasters and supertasters (Bartoshuk *et al.* 1994). The estimated Caucasian distribution is

approximately 30% non-tasters and 70% tasters (of which ~25% are supertasters) (Bartoshuk *et al.* 1994).

The variation seen in the human population to the bitterness elicited by PTC and PROP appears to be linked to genetic variation on the TAS2R38 gene. Single nucleotide polymorphisms in this gene result in 5 common haplotypes which account for 55%-85% of the variance in PTC sensitivity (Kim *et al.* 2003; Reed *et al.* 2006). The taster haplotype is defined by the three variants: proline-alanine-valine (PAV). The non-taster haplotype was defined by the three variants: alanine-valine-isoleucine (AVI). A familial study of a large Sardinian cohort (Prodi *et al.* 2004), suggested those who were homozygotous for the recessive allele (AVI/AVI) were non-tasters, whilst possession of one or two dominant alleles (PAV) were tasters with varying degrees of sensitivity to PROP.

Taster status has also been correlated with anatomical differences in tongue papillae. Tasters tend to have a larger number of fungiform papillae than non-tasters, with supertasters having the highest density (Miller *et al.* 1990; Bartoshuk *et al.* 1994; Delwiche *et al.* 2001; Yackinous *et al.* 2002).

PROP tasters have been reported to be more sensitive to a range of other bitter compounds. Ly and Drewnowski (2001) noted PROP tasters rated the bitterness of caffeine solutions as more intense than non-tasters, corroborating findings by Hall *et al* (Hall *et al.* 1975) suggesting PTC tasters were more sensitive to caffeine bitterness. Similar findings have been reported for sodium benzoate, urea and quinine (Leach *et al.* 1986; Bartoshuk *et al.* 1988; Mela 1989). However, there is also a large body of evidence suggesting PROP sensitivity does not predict sensitivity to all bitter compounds (Schifferstein *et al.* 1991; Smagghe *et al.* 1998; Keast *et al.* 2003). The conflicting results may be partly attributable to differences in methods used for categorisation of individual's taster status (threshold detection methods, responses to PROP impregnated paper, PROP/NaCL ratio scores).

PTC or PROP taster status has been suggested to confer sensitivity to other taste qualities, a finding which may relate to the modified oral anatomy between taster groups (Delwiche *et al.* 2001; Yackinous *et al.* 2002). Bartoshuk (1979) and Gent and Bartoshuk (1983), found PROP tasters rated sweetness of both sucrose and an intense sweetener, neohesperidin dihydrochalcone (NHDC), as higher than non-tasters. However, Ly and Drewnowski (2001) reported no significant differences in sweetness ratings between PROP taster groups, but found NHDC suppressed the bitterness of caffeine more effectively in PROP tasters than non-tasters. Similarly, evidence that PROP tasters display enhanced sensitivity for acids has been reported (Prutkin, 1999).

Prescott *et al* (2004) investigated the influence of PROP taster status in responses to carbonated fruit drinks. These authors found supertasters rated intensity of sourness and irritation higher than non-tasters, although differences in ratings of sweetness were not significant. PROP taster status has also been linked to sensitivity to oral irritation by capsaicin (Prescott *et al.* 2000).

Taken together, these data provide some indication that PROP taster status may indicate sensitivity to oral stimuli. However, results from different research groups are often conflicting, and the method of classification of taster status not fully standardised, consequently drawing definitive comparative conclusions is problematic. It would seem prudent in light of the evidence reported regarding bitter sensitivity, and specifically caffeine sensitivity (Hall *et al.* 1975; Smagghe *et al.* 1998), to identify panellist's PROP taster status prior to undertaking sensory evaluation of products containing bitter tastants.

5.1.2. Tastant and aroma interactions of bitter compounds

5.1.2.1. Bitter and sweet interactions

Research by Pangborn (1960) focussed on taste interrelationships and provided evidence of a reciprocal suppressive effect of sweet (sucrose) and bitter (caffeine) tastants in mixtures. Subsequent studies have confirmed the suppressive effects of sweeteners on bitterness intensity (Kamen *et al.* 1961; Calvino *et al.* 1990; Keast *et al.* 2003; Mojet *et al.* 2004). However, Schiffman *et al* (1986) investigated the influence of caffeine on both natural and artificial sweeteners and suggested caffeine was able to intensify the taste of sweeteners which carried an inherent bitter taste. This enhancement was evident for both the sweetness and bitter taste components of the artificial sweeteners, potentiating the overall taste intensity. This effect of caffeine was absent when the sweeteners examined lacked a bitter taste component (sucrose, fructose and aspartame).

Tastant concentration may influence the effects of bitter compounds on sweetness perception, Calvino *et al* (1990) reported a greater suppression of sweetness by caffeine at low sucrose levels. Similarly, Kamen *et al* (1961) noted a trend for suppression of sweetness by caffeine although this did not reach significance at the levels examined (up to 0.093g/L, or 0.5mM ,caffeine).

5.1.2.2. Bitter and sour interactions

Attempts to elucidate the relationship between bitter and sour compounds have yielded variable data. Kamen *et al* (1961) found mixtures of caffeine and citric acid resulted in the enhancement of sourness and bitterness ratings. Conversely, Pangborn (1960) reported contrary findings i.e. suppressive effects of citric acid on bitterness and caffeine on sourness. These conflicting results may be a consequence of concentrations of the tastants examined: in Pangborn's study subthreshold concentrations of both

caffeine and citric acid were investigated, whilst Kamen (1961) used suprathreshold concentrations.

An overview of binary taste interactions by Keast (2003) provided schematics reviewing sweet, sour, bitter, and salty interactions. These authors noted at low intensity, mixtures of sour and bitter compounds were mutually enhancing whilst at higher intensities sourness was suppressed by bitter taste and bitterness was variably affected by sour taste.

5.1.2.3. Bitter taste and flavour

Bitter taste is an integral component of flavour profiles of many products such as coffee, chocolate, beer and wine. Current literature details only direct influence of bitter tastants on aroma compounds.

There is evidence suggesting the bitter tastant caffeine, may form complexes with certain aroma volatiles, thereby increasing their solubility and possibly resulting in a decreased headspace volatile concentration and perception (King *et al.* 1982). However, the concentrations of caffeine (>20mM) used to produce this effect is far higher than levels in soft-drinks (≤0.7mM). Charles-Bernard *et al* (2005) used lower levels of caffeine (≤2.5mM) to examine interactions between non-volatile and volatile components of coffee. Results found no significant interactions of caffeine with the selected volatile compounds. It is unlikely therefore, that at standard beverage concentration, any physicochemical effect of caffeine on volatile components would be significant.

Nevertheless, Opet *et al* (1990) demonstrated an increase in overall intensity of menthol on addition of caffeine (2g/L or ~10mM) and time-intensity data suggested this enhancement may be more prominent over the time course of menthol perception.

Interestingly, Labbe *et al* (2006) reported an increase in bitter intensity when caffeine was paired with a cocoa flavouring but also noted a non-significant

trend for enhancement of bitterness of a caffeinated milk beverage on addition of a vanilla flavouring, thereby suggesting aroma may influence bitter taste perception.

5.1.3. Rationale for inclusion of caffeine in carbonated beverages

Caffeine (1,3,7-trimethylxanthine) is a bitter taste compound, naturally occurring in a number of food and drink products such as cocoa, chocolate, tea and coffee. Furthermore, caffeine is an added ingredient in over 70% of carbonated soft drinks (Griffiths *et al.* 2000). Justification for this inclusion of caffeine in beverages relies on the concept that caffeine is a flavouring agent and plays an integral role in the flavour profile.

Despite evidence to indicate caffeine potentially complexes with certain aroma volatiles (King *et al.* 1982), reports suggest it may have little effect on perception of flavour at levels commonly encountered in soft drinks. Findings of Griffiths and Vernotica (2000), supported by recent data from Keast and Riddell (2006), indicated both consumers and trained sensory assessors were unable to distinguish between caffeinated and non-caffeinated cola flavoured beverages. The study by Griffiths and Vernotica (2000), demonstrated that the concentration of caffeine required for significant differences to be detected was 0.2mg/ml (>1mM), much higher than is commonly included in carbonated soft drinks. It is possible that other interactions within a complex carbonated soft-drink matrix may mask any subtle effects of caffeine, reducing the discrimination ability. Trained assessors were able to detect differences in flavour when the same concentration of caffeine was included in aqueous sugar solutions but not in cola beverages (Keast 2006), providing some support for this theory.

Absolute amounts of caffeine found in common beverages vary significantly. Filtered coffee contains 85mg, tea contains 45-55mg per cup and cola 45mg per can. Recent trends, however, have seen an increase in products containing elevated amounts of caffeine and marketed as 'energy' or

'stimulant' drinks, such as Red Bull and V, aimed at the 18-35 year age group. These products contain up to 80mg caffeine per serving (0.32mg/ml or 1.65mM) and the increase in caffeine concentration compared to standard carbonated soft drinks may influence taste and/or flavour quality to a greater degree. Nonetheless, the major selling point of the elevated caffeine concentration is its beneficial influence on performance and alertness.

5.1.4. Physiological role of caffeine

Numerous studies (for review see Smith (2002)) have examined the physiological influence of caffeine and found effects on attention, alertness, mental and physical performance. Caffeine, even in low doses found in commercial products, has been shown to improve mood, increase alertness and reduce fatigue (Lieberman *et al.* 1987; Warburton 1995; Smith *et al.* 1999; Warburton *et al.* 2001) although high doses (300mg or more) can lead to anxiousness in some individuals. Additionally, caffeine is known to be mildly addictive and caffeine consumers may suffer symptoms including headaches, feelings of drowsiness, fatigue and work difficulty, depression and irritability, when they stop consuming caffeine (Keast 2006). Interestingly, research by Yeomans *et al* (2001; 2005) suggests caffeine may influence product liking and preference, especially when consumers are in a caffeine-deprived state.

Studies report a beneficial impact of caffeine on reaction times (Smith *et al.* 1977; Lieberman *et al.* 1987; Smith *et al.* 1999), memory (Smith *et al.* 1999), and real-life paradigms such as driving (Regina *et al.* 1974; Brice *et al.* 2001). In addition, regular low dose caffeine consumption, not only larger dose single intake, appears to result in improved performance (Jarvis 1993).

5.1.5. Effects of combination of glucose and caffeine in beverages

Studies investigating the effects of 'energy' drinks indicate significant improvements in cognitive performance and alertness (Alford *et al.* 2001; Kennedy *et al.* 2004; Scholey *et al.* 2004; Scholey *et al.* 2004; Smit *et al.*

2004). The most common constituents of these drinks are caffeine and a carbohydrate source, usually glucose. Most studies have focussed on an evaluation of the whole product but a minority have attempted to disconnect effects due to the separate components. Of these studies, data suggests caffeine may be the main constituent responsible for performance improvements (Smit *et al.* 2004).

Smit *et al* (2004) reported energetic arousal and reaction time benefits were attributable to caffeine but noted minor effects of carbohydrate, most noticeably on mood related constructs. Some agreement was reported by Scholey and Kennedy (2004) who noted a trend towards improvements in memory and attention of a caffeine only energy drink fraction. However, these authors concluded neither caffeine nor glucose in isolation resulted in significant improvements. Data indicated significant improvements in performance resulted from consumption of the complete energy drink raising the possibility that the combination of the two factors resulted in a synergistic enhancing effect.

5.1.6. Increasing complexity of the model beverage system

It would appear from the evidence presented that caffeine has an integral role in performance enhancement associated with consumption of 'energy' drinks. What is not clear, however, is the impact of inclusion of caffeine on the perception of overall flavour and individual taste quality of a beverage. Studies investigating caffeinated and non-caffeinated cola beverages suggest consumers are unable to perceive any difference between the two until caffeine concentrations are approximately double those found in standard commercial soft-drinks. With the increasing consumer market for highly-caffeinated and glucose-containing 'energy' drinks, the concentration of caffeine at these levels may significantly influence the product flavour profile. In addition, 'energy' drinks are not traditionally cola-flavoured but more often fruit-flavoured. The use of different aroma volatiles may also influence the perception of caffeine-induced bitter taste as indicated by data

from Labbe et al (2006) showing perception of bitterness was significantly enhanced by a cocoa flavouring but not by vanilla. Congruency of the bitter-aroma pairing may therefore play a role in the enhancement, as a cocoa flavouring, rather than vanilla, may be more perceptually associated with a bitter taste (e.g. as in chocolate).

In order to more fully understand the influence of caffeine on the perceptual profile of a beverage system, the complexity of the citrus-flavoured carbonated model system employed to study effects of sugars, acids and carbonation was increased. Whilst retaining glucose, citric acid and carbonation as design factors, caffeine was now also included. Effects of caffeine were examined at a concentration range encompassing levels found commonly in standard soft-drinks and higher caffeine-containing 'energy' drinks.

5.2. Materials and methods

5.2.1. Sensory panel

A total of 10 assessors (2 male, 8 females, aged between 43-68yrs) from the University of Nottingham external sensory panel, were invited to take part in the study. All panellists had previously participated in the sensory evaluation of carbonated beverages (Chapter 4).

Full approval of the University of Nottingham Ethics Committee was sought and obtained for the project.

5.2.1.1. PROP taster status

Panellists had been pre-screened for PROP sensitivity as part of UoN panel monitoring protocols. PROP taster status was determined by rating the intensity of PROP impregnated filter paper on a labelled magnitude scale (LMS) (Bartoshuk *et al.* 2004) and additionally, an assessment of fungiform papillae numbers had been undertaken. Panellists were termed either PROP

non-tasters or PROP tasters on the basis of these results. All panellists included in this study were determined to be PROP tasters but no attempt was made to further sub-divide the taster group in tasters and supertasters.

5.2.2. Experimental model design space

A D-optimal design (created in Design Expert software, Stat-Ease Inc, Minneapolis) was constructed using glucose (0-150g/L), citric acid (0-1.5g/L) and caffeine (0-0.2g/L) as numerical factors and carbonation as a categorical factor (nominally; none and high). Volatile level (2.5ppm citral and 2.5ppm limonene) was constant for all samples.

A total of 20 samples from within the design space (including 3 replicate and 3 lack of fit points) were assessed by sensory profiling, using attributes previously generated in profiling of carbonated beverages (Chapter 4). The composition of these samples is described in Table 5.2:1.

Table 5.2:1: Composition of samples in model design

sample	glucose g/L	citric acid g/L	caffeine g/L	carbonation level
1	150.00	1.50	0.00	none
2	150.00	1.50	0.20	none
3	150.00	0.00	0.00	high
4	0.00	0.00	0.00	none
5	75.00	1.50	0.00	high
6	0.00	1.50	0.20	high
7	0.00	0.00	0.10	high
8	75.00	0.75	0.10	none
9	37.50	1.13	0.10	high
10	37.50	1.13	0.10	high
11	150.00	0.00	0.20	none
12	112.50	0.38	0.10	high
13	150.00	0.75	0.20	high
14	0.00	0.75	0.00	high
15	0.00	1.50	0.00	none
16	75.00	0.00	0.20	high
17	150.00	1.50	0.10	high
18	150.00	0.00	0.20	none
19	0.00	1.50	0.00	none
20	0.00	0.00	0.20	none

5.2.3. Sample preparation and presentation

Samples were prepared in mineral water (Brecon Carreg, U.K.) using glucose (99+%, Fisher Scientific, U.K.), citric acid (99%, Lancaster Synthesis, U.K.) and caffeine (98+%, Sigma Aldrich, Germany) as indicated by the model design. To each sample, aroma volatiles were added to obtain a final concentration of 2.5ppm for both citral and limonene (Aldrich, U.K.). All samples were roller bed mixed for a minimum of 1hr before refrigeration (4-6°C).

Samples were carbonated as described in section 2.2.2. Immediately after carbonation, all samples (both carbonated and non-carbonated) were decanted into glass vials (35ml), minimising free headspace, securely sealed and refrigerated until use.

Samples were presented monadically, in a balanced order, randomised across panellists. Panellists were presented samples in sets of three with a 15min break between sets to reduce sensory fatigue. Panellists were instructed to palate cleanse with the cracker and mineral water (Brecon Carreg, U.K.) provided at the beginning of each sample set and between samples within a set.

5.2.4. Sensory evaluation

Sensory profiling of the samples chosen from the design space was undertaken using the trained panel of assessors as in Chapter 4. All samples (20) were evaluated within one session and replicate measures were obtained over 2 further sessions (3 replicates in total).

Principal Component Analysis performed on data obtained from profiling of carbonated model beverages (Chapter 4, section 4.3.3) suggested that both the drying and acidic aftertaste attributes were highly correlated with drying in-mouth and acidic taste. As scoring of these aftertaste attributes appeared to yield no further data to discriminate between samples they were removed from this study. In addition, a new attribute, bitterness, was included due to the addition of caffeine in the model beverage system. The removal of the 2 aftertaste attributes allowed addition of this new attribute without subjecting panellists to greater fatigue.

Panellists were re-familiarised with both the previously generated attributes and the agreed testing protocols. Full testing methodology and protocols are detailed in Chapter 4, section 4.2. A full list of attributes, together with agreed definitions and verbal scale anchors, used in the current study is included in Table 5.2:2:.

Table 5.2:2: Profile attributes, agreed definitions and scale anchors

ATTRIBUTE	DEFINITION	SCALE
overall impression of fizziness	*overall perception in the whole mouth including both bubbling feeling and pain perception*	LOW---HIGH
tingling	*sensation associated with fizz/acidity on tongue and around inside of mouth – like a pins and needles sensation*	LOW---HIGH
drying/astringent	*tactile sensation due to shrinking, drawing or puckering of oral epithelium*	NOT---VERY
irritant/burning (chemical)	*Irritating/burning sensation which lingers after the stimulus is removed*	LOW---HIGH
citrus-like flavour	*lemon/lime/citrus flavour*	LOW---HIGH
sweetness	*taste stimulated by sugar in water*	NOT---VERY
sourness	*taste stimulated by acid in water*	NOT---VERY
bitterness	*taste stimulated by caffeine in water*	NOT---VERY
bitter aftertaste	*aftertaste stimulated by caffeine in water*	NOT---VERY

5.2.5. Data analysis and panel performance monitoring

In common with previous studies (Chapters 3 and 4), panel performance was monitored by assessment of replicate scores. Using Fizz software (Biosystemes, France) the ability of individual panellists to reliably and repeatability rate the attributes under investigation across sessions was

assessed using the coefficient of variance (CV) measures resulting from one-way ANOVA. Individual panellist's ability to discriminate between the samples for each of the attributes scored was determined by assessment of the probability value, FPROD, from the ANOVA result. Plots of CV versus FPROD (probability of discriminating between samples) were used to visualise panel performance data.

Two-way ANOVA (analysis by attribute with product and judge factors), and where appropriate, Tukey's HSD multiple comparison test were performed on the mean panel data to determine significant differences between samples for each of the assessed attributes.

As in the previous chapter, PCA with varimax rotation was used to determine how the attributes discriminated between the samples of the Model (XLSTAT version 7.5.2, Addinsoft, USA).

5.2.5.1. Generation of Predictive Models

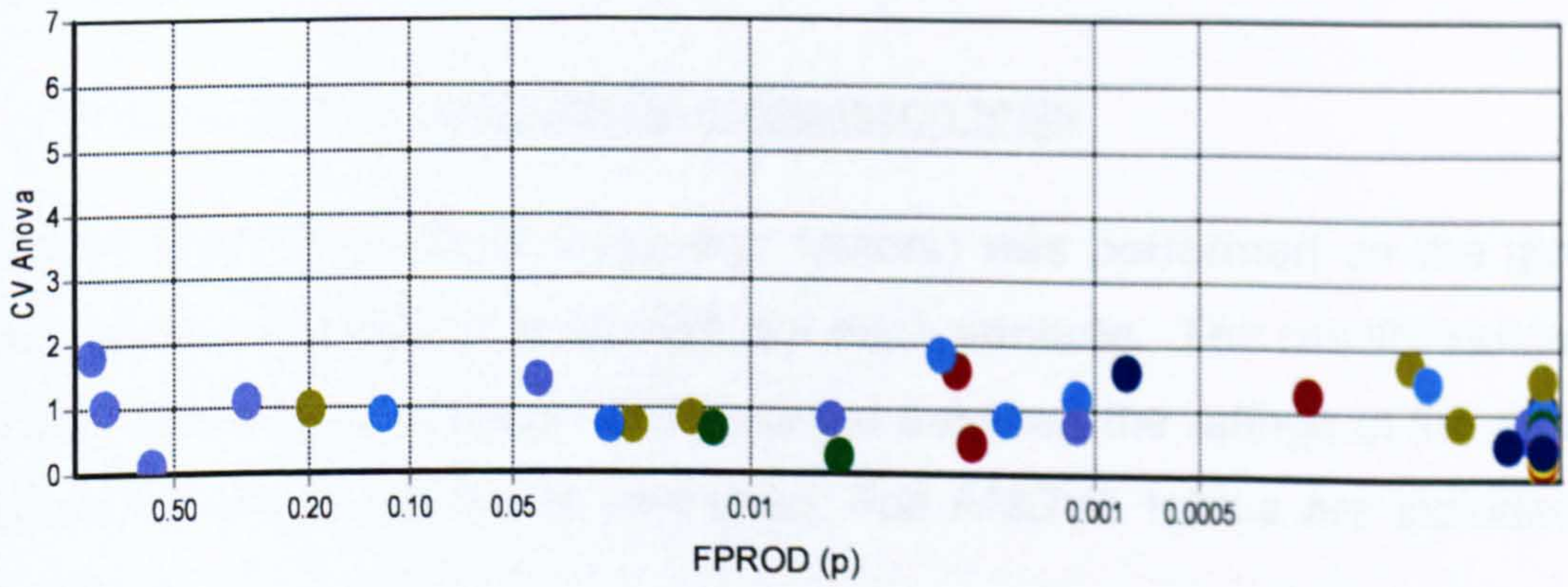
Predictive polynomial models were generated to explain variations in perception of each attribute as a function of the concentration of glucose, citric acid, caffeine and the presence of carbonation. As previously described (Chapters 3 and 4), non-significant terms, as determined by ANOVA, were removed and a final mathematical model was chosen which best represented the data.

The ability of the final model to explain the data was indicated by adjusted R^2 and predictive R^2 values. The predictive ability of these models was assessed by means of the evaluation of a separate set of samples taken from within the design space but not included as part of the data used to generate the predictive models.

5.3. Results

5.3.1. Assessment of panel performance

Assessment of panel performance was carried out as previously described in Chapter 3, section 3.3.1. ANOVA derived variation coefficients for each panellist were obtained and used as a measure of individual's repeatability in scoring of samples across the 3 replicates. The probability (p value), defined in terms of FPROD, obtained from the one-way ANOVA was used as a measure of discrimination.



Panel monitoring data showing coefficient of variance (CV) plotted against discrimination probability (FPROD). Data points are colour coded for attributes and each data point represents a panellist's mean result (3 replicates)

Figure 5-1: Panel monitoring; repeatability and discrimination

Figure 5-1 shows measures of discriminative ability (FPROD) and repeatability (CV) for the whole panel. Assessment of panel performance indicated all panellists displayed an acceptable level of repeatability and discriminative ability.

However, in concurrence with findings from previous profiling of carbonated beverages (Chapter 4), a proportion of panellists were unable to discriminate significantly at the 10% confidence level ($p<0.1$) between samples by scoring the attribute 'citrus-like flavour'. As mentioned previously (Chapter 4, section 4.3.2), the aroma volatiles, citral and limonene, are incorporated at a constant concentration within all samples. It is, therefore, not surprising that this attribute is problematic in enabling discrimination between samples. It should be noted, however, that panellist's scoring of this attribute indicated good repeatability and the majority were able to significantly discriminate between samples ($p<0.05$) which implies interaction of other constituents influences flavour perception.

5.3.2. ANOVA and multiple comparison tests

Two-way ANOVA (product and judge factors) was performed on the global mean of the panel data (3 replicates) for each attribute. The results indicated that significant differences ($p<0.05$) existed between the ratings of the design samples for all the attributes examined. Full ANOVA tables are included in Appendix 4.

As in previous studies (Chapter 2 and 3), results from ANOVA from a number of attributes showed significant judge and product-judge interactions. Again, this is mainly attributable to the large number of samples within the model design ($n=20$) and the similarity of many samples in terms of assessed attributes leading to cross-over interactions between panellists. After consideration of raw data, these interactions were not deemed to reflect poor panel performance.

The panel mean values, standard deviations and results of multiple comparison analysis are shown in Table 5.3:1. Multiple comparison tests indicated a wide variation in sample groupings across the attributes. The mouthfeel attributes 'fizziness', 'tingling' and 'irritant', were able to separate the samples into a small number of well defined groups (3-4). The attributes

'sweetness' and 'sourness', also clearly separated the samples but into a larger number (7) of defined groups suggesting variation of sample composition had a greater effect on these attributes. Multiple comparison analysis of scoring of attributes 'drying', 'citrus flavour', 'bitterness' and 'bitter aftertaste', again resulted in a larger number of sample groupings (up to 10 groups). In contrast to other attributes, however, these showed greater overlap of groupings between the samples (Table 5.3:1) indicating difficulty in discriminating between sub groups of samples.

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Table 5.3:1: Mean panel scores and post hoc groupings

	Attribute								
	Fizziness	Tingling	Drying-astringent	Irritant	Citrus flavour	Sweetness	Sourness	Bitterness	Bitter aftertaste
Sample	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)
1	0.29 *0.4* C	0.33 *0.5* D	3.49 *2.0* GH	0.45 *0.7* C	6.68 *2.8* A	7.34 *2.4* B	3.85 *2.4* DE	2.19 *1.9* H	3.08 *2.0* G
2	0.13 *0.1* C	0.16 *0.2* D	3.77 *2.3* FGH	0.76 *1.6* C	6.61 *2.5* A	6.44 *2.9* BC	3.71 *2.2* DE	4.41 *2.9* BCDEF	5.13 *2.6* BCDEFG
3	6.48 *1.8* AB	6.83 *1.9* ABC	5.09 *2.0* DEF	5.75 *2.8* AB	5.35 *2.7* ABCD	6.53 *2.5* BC	2.51 *1.9* EF	3.44 *2.1* EFGH	4.46 *2.6* DEFG
4	0.06 *0.1* C	0.08 *0.1* D	0.98 *2.1* J	0.26 *0.4* C	4.32 *3.3* BCD	0.11 *0.2* H	1.02 *1.4* FG	2.46 *3.1* GH	3.28 *3.3* G
5	7.48 *1.4* A	8.02 *1.5* A	6.72 *1.8* ABC	6.01 *2.7* AB	5.83 *2.4* ABC	2.98 *1.9* E	6.53 *1.8* BC	3.27 *1.9* FGH	4.35 *2.1* DEFG
6	7.35 *2.0* A	7.73 *1.8* AB	7.64 *2.3* A	6.47 *2.8* A	3.53 *1.9* D	0.30 *0.4* H	7.63 *2.2* AB	6.80 *2.2* A	7.75 *1.7* A
7	6.68 *1.7* AB	6.71 *1.8* BC	6.55 *2.5* ABCD	5.42 *3.0* AB	3.82 *2.4* CD	0.29 *0.5* H	3.94 *2.2* DE	6.32 *2.2* AB	7.05 *2.0* AB
8	0.19 *0.4* C	0.17 *0.3* D	2.51 *1.7* HI	0.18 *0.2* C	5.77 *2.5* ABC	3.36 *2.1* E	2.96 *1.7* DE	3.66 *2.4* EFGH	4.40 *2.2* DEFG
9	6.76 *1.8* AB	7.35 *1.7* ABC	6.71 *1.8* ABC	5.80 *2.8* AB	4.72 *2.3* ABCD	1.71 *1.5* F	6.35 *1.8* BC	4.89 *1.9* ABCDEF	5.78 *1.8* ABCDE
10	6.89 *2.0* AB	7.40 *1.5* ABC	7.39 *1.8* AB	6.35 *2.6* A	4.84 *2.4* ABCD	1.41 *1.1* FG	5.83 *1.5* C	5.93 *2.4* ABCD	6.95 *2.0* AB
11	0.08 *0.1* C	0.11 *0.1* D	1.77 *2.3* IJ	0.60 *1.2* C	5.73 *2.8* ABC	8.75 *1.3* A	0.67 *1.0* G	3.02 *2.7* FGH	3.53 *2.6* FG
12	5.97 *2.0* B	6.43 *2.0* C	4.93 *1.8* EFG	4.85 *2.3* B	5.93 *2.5* AB	5.02 *2.1* D	3.05 *1.6* DE	4.27 *2.5* CDEFG	5.01 *2.7* BCDEFG
13	6.70 *2.0* AB	7.02 *1.9* ABC	5.96 *1.9* BCDE	6.17 *2.5* AB	5.97 *2.7* AB	5.97 *2.9* CD	2.95 *1.8* DE	5.22 *2.1* ABCDE	6.35 *2.5* ABCD
14	6.48 *1.6* AB	6.77 *1.6* ABC	7.12 *1.9* ABC	6.00 *2.6* AB	3.49 *2.0* D	0.45 *0.5* GH	6.36 *2.1* BC	6.01 *2.8* ABC	6.62 *1.9* ABC
15	0.18 *0.3* C	0.22 *0.4* D	5.81 *3.6* CDE	0.30 *0.4* C	3.63 *2.4* D	0.14 *0.2* H	8.38 *1.5* A	3.59 *2.4* EFGH	4.20 *2.0* EFG
16	6.64 *1.7* AB	6.67 *2.0* BC	6.30 *1.6* ABCDE	5.65 *2.6* AB	5.79 *2.5* ABC	3.77 *2.1* E	2.81 *1.7* DE	6.15 *2.0* ABC	7.14 *1.8* AB
17	5.98 *1.5* B	6.51 *1.6* BC	6.24 *2.1* ABCDE	4.82 *2.1* B	6.08 *2.9* AB	5.74 *2.9* CD	4.13 *2.6* D	4.52 *3.0* BCDEF	5.45 *2.7* BCDEF
18	0.10 *0.1* C	0.15 *0.1* D	1.38 *1.8* IJ	0.98 *1.9* C	5.47 *2.8* ABCD	8.59 *1.2* A	0.84 *1.0* G	3.31 *3.1* EFGH	3.44 *2.7* FG
19	0.13 *0.2* C	0.12 *0.2* D	5.68 *2.6* CDE	0.38 *0.7* C	4.11 *2.4* BCD	0.25 *0.5* H	8.37 *1.5* A	4.06 *2.7* DEFGH	4.74 *2.6* CDEFG
20	0.05 *0.1* C	0.08 *0.1* D	1.69 *2.3* IJ	0.64 *1.0* C	3.83 *3.5* CD	0.11 *0.2* H	0.63 *0.9* G	6.54 *3.2* A	6.85 *3.1* ABC

standard deviations are shown in italics

samples with the same letter are not significantly different from each other (Tukey's HSD)

5.3.3. Principal component analysis

Analysis of the panel data using PCA found over 96% of the variation within the data could be accounted for by 3 principle components (axes). The attribute correlations and % variance explained by each PC are detailed in Table 5.3:2. Bi-plots displaying both the attributes and the sample loadings on the PCs are shown in Figure 5-2 (PC 1 vs PC2) and Figure 5-3 (PC1 vs PC3).

Table 5.3:2: PCA attribute correlations and % variance explained by each principle component axis

Attributes	PC 1 (60%)	PC 2 (20%)	PC 3 (16%)
fizziness	**0.982**	-0.061	0.163
tingling	**0.980**	-0.050	0.172
drying	**0.721**	-0.180	0.640
irritant	**0.986**	-0.058	0.136
citrus flavour	0.020	**0.845**	-0.187
sweetness	-0.040	**0.938**	-0.304
sourness	0.150	-0.266	**0.948**
bitterness	0.557	**-0.610**	-0.074
bitter AT	**0.647**	-0.571	-0.015

In common with the previous study (Chapter 4), results indicated mouthfeel attributes accounted for most of the variation between the samples explained by principle component (PC) 1. 'Fizziness', 'tingling', 'drying' and 'irritant' attributes all had correlations above 0.7 on this axis, and the bi-plots (Figure 5-2 and Figure 5-3) show samples distributed across this axis. The attributes 'bitterness', 'citrus flavour' and 'sweetness' characterised PC2 (Table 5.3:2), whilst 'sourness' correlated highly with PC3. Examination of bi-plots shown in Figures 2 and 3 demonstrated that, as for the previous study, mouthfeel attributes appeared to be related to carbonation level. In the absence of the low level of carbonation from this study, the spread of samples along PC1 is clear; noncarbonated samples are in the left hand portion of the plot and

carbonated samples in the right hand portion (Figure 5-2). Separation along PC2 was related to glucose concentration of samples; glucose content decreased in samples spreading from top to bottom of the bi-plot (Figure 5-2).

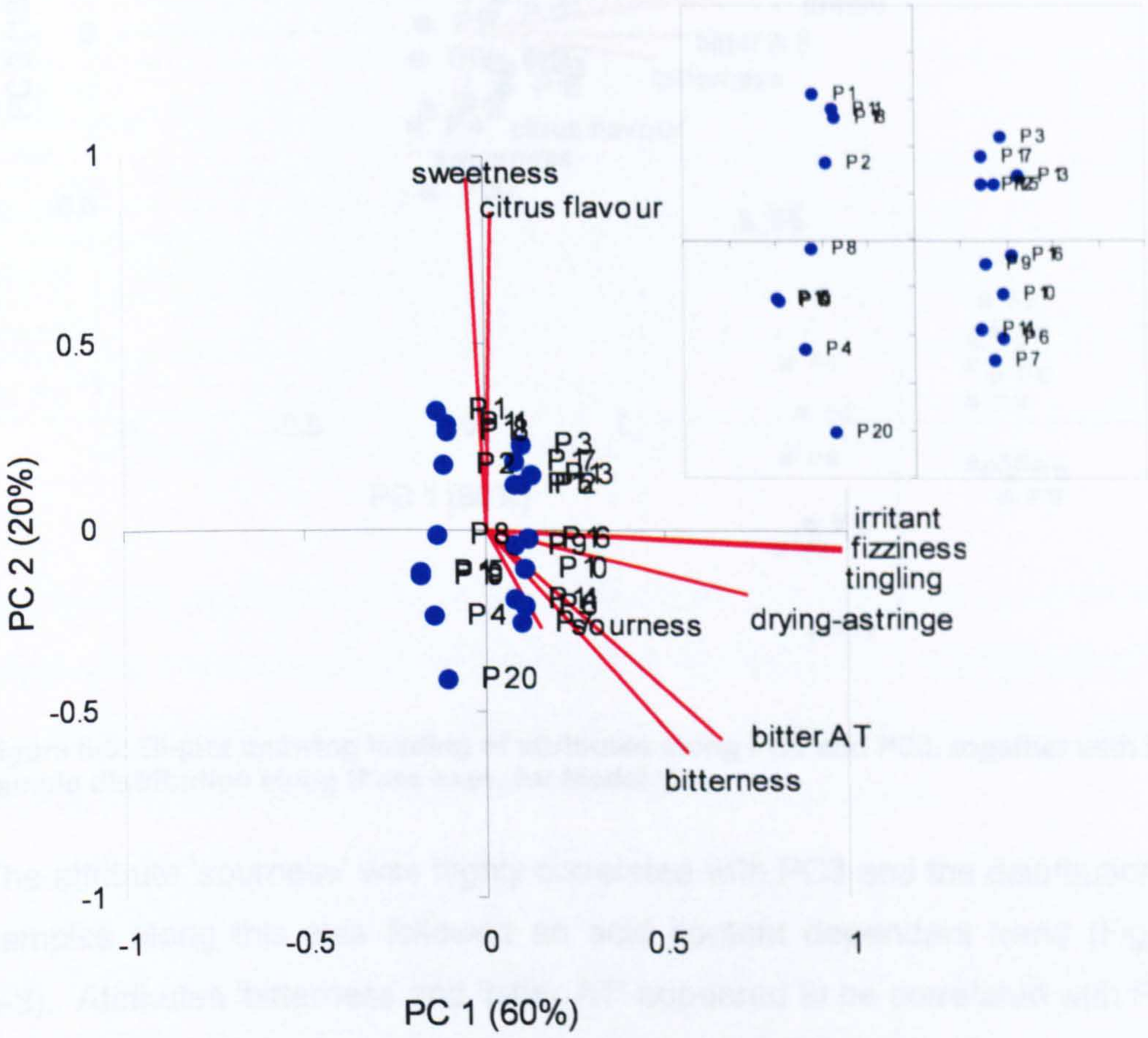


Figure 5-2: Bi-plot showing loading of attributes along PC1 and PC2, together with the sample distribution along these axes, for Model G.

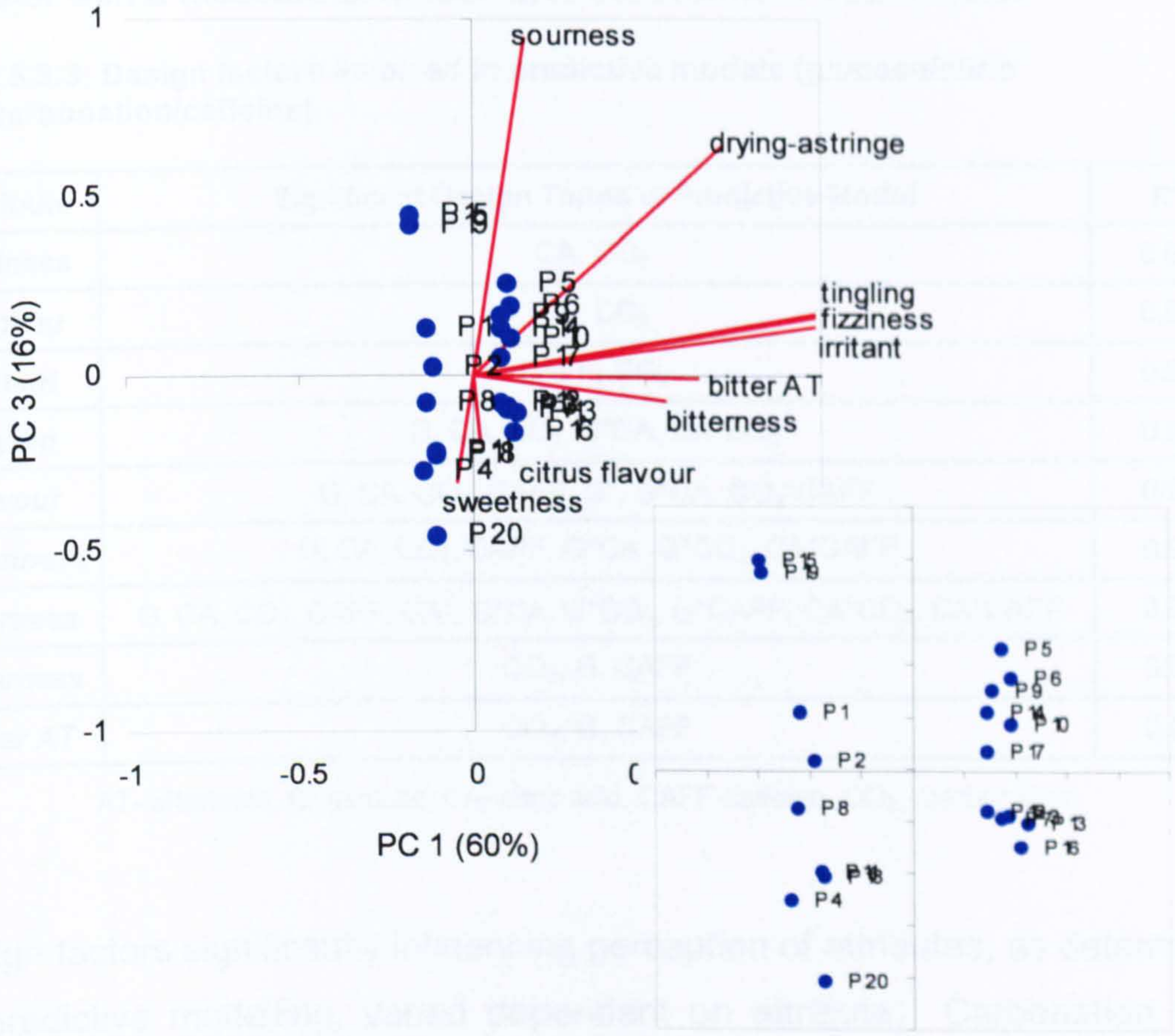


Figure 5-3: Bi-plot showing loading of attributes along PC1 and PC3, together with the sample distribution along these axes, for Model G .

The attribute 'sourness' was highly correlated with PC3 and the distribution of samples along this axis followed an acid content dependant trend (Figure 5-3). Attributes 'bitterness and 'bitter AT' appeared to be correlated with PC1 and PC2 (Table 5.3:2), but the distribution of samples along these axes was not clearly associated with caffeine content.

5.3.4. Predictive models

Using the global mean of the panellists score for each attribute, significant polynomial predictive models were generated using multiple linear regression (Design Expert). These models described the perceptual results in terms of the design factors used; concentration of glucose, citric acid, caffeine and presence of carbonation, for each attribute assessed. A summary table

detailing design factors included as significant terms in the predictive models, *together with a measure of model fit, R^2, is shown in* Table 5.3:3.

Table 5.3:3: Design factors involved in predictive models (*glucose/citric acid/carbonation/caffeine*).

Attribute	Significant Design Terms In *Predictive Model*	R^2
fizziness	CA, CO_2	0.99
tingling	CA, CO_2	0.99
irritant	CAFF, CO_2	0.98
drying	G, CA, CO_2, G*CA, CA*CO_2	0.96
flavour	G, CA, CO_2, CAFF, G^2, G*CA, CO_2*CAFF	0.98
sweetness	G, CA, CO_2, CAFF, G*CA, G*CO_2, CA*CAFF	0.99
sourness	G, CA, CO_2, CAFF, CA^2, G*CA, G*CO_2, G*CAFF, CA*CO_2, CA*CAFF	0.99
bitterness	CO_2, G, CAFF	0.83
bitter AT	CO_2, G, CAFF	0.84

AT- aftertaste, G- glucose, CA- citric acid, CAFF-caffeine, CO_2 - carbonation

Design factors significantly influencing perception of attributes, as determined by predictive modelling, varied dependant on attribute. Carbonation level appeared to significantly influence all attributes examined, glucose, citric acid and caffeine concentration modified 6 out of the 9 attributes.

The summary table, Table 5.3:3, can be used for rapid identification of significant design factors and interaction terms for each attribute together with measures of goodness of model fit. Nonetheless, for full analysis detailing the weightings of each factor the reader is directed to Table 5.3:4. This table displays the full predictive equations generated, in actual terms of design factors, and includes PRESS statistics, adequate precision and R^2 terms for each attribute model.

Table 5.3:4: Predictive equations (in actual factors) generated for design attributes (sqrt-square root)

				significant model terms								model statistics			
attribute	CO_2* Level		intercept	glucose	citric acid	caffeine	glucose^2	citric acid^2	gluc*acid	gluc*caffeine	acid*caffeine	PRESS	Adj R^2	Pred R^2	Adeq Precision
overall fizziness	none	sqrt(fizz)=	0.29		+ 0.08							0.15	0.99	0.99	75.68
	high		2.52		+ 0.08										
tingling	none	sqrt(ting)=	0.32		+ 0.09							0.13	0.99	0.99	83.31
	high		2.58		+ 0.09										
drying in mouth	none	drying=	1.62	- 2.97	+ 2.65				-6.58^{-3}			0.40	0.98	0.97	36.33
	high		6.04	- 2.97	+ 1.20				-6.58^{-3}						
irritant	none	sqrt(irritant)=	0.6			+0.86						6.76	0.95	0.93	25.08
	high		2.3			+0.86									
citrus flavour	none	citrus flavour=	4.23	+ 0.04	- 0.26	- 1.52	-1.67^{-4}		$+5.25^{-3}$			0.98	0.97	0.95	30.10
	high		3.69	+ 0.04	- 0.26	+ 1.03	-1.67^{-4}		$+5.25^{-3}$						
sweetness	none	$(sweetness)^{0.81}$=	8.47^{-3}	+ 0.04	+ 0.19	+ 1.25			-3.45^{-3}		-2.12	0.71	0.99	0.99	72.94
	high		0.39	+ 0.03	+ 0.19	+ 1.25			-3.45^{-3}		-2.12				
sourness	none	sourness=	1.25	-6.39^{-3}	+ 3.85	- 3.66		+ 0.58	- 0.02	$+3.82^{-2}$	- 2.33	3.75	0.99	0.97	46.88
	high		4.07	- 0.01	+ 2.51	- 3.66		+ 0.58	- 0.02	$+3.82^{-2}$	- 2.33				
bitterness	none	bitterness=	3.65	- 0.01		+ 10.0						10.46	0.80	0.72	19.23
	high		5.08	- 0.01		+ 10.0									
bitter aftertaste	none	bitter aftertaste=	4.28	- 0.01		+9.4						10.46	0.81	0.74	19.27
	high		6.03	- 0.01		+9.4									

sqrt=square root

***values differing between none and high CO_2 signify a significant contribution of CO_2 level on the predictive model for the attribute**

Interaction and contour plots can be used to visualise the data to clarify the influence of varying the concentration of each design factor. These plots, together with data from Table 5.3:3 Table 5.3:4 are used to investigate the impact of each design factor, on the attributes rated, in the following sections.

5.3.4.1. Mouthfeel attributes

In agreement with previous findings (Chapter 4), carbonation level was the main influencing factor for perception of 'overall fizziness' and 'tingling' attributes (Figure 5-4). Increasing concentration of citric acid resulted in a small enhancement of both 'fizziness' and 'tingling'. Caffeine did not appear to influence either attribute significantly at the concentrations examined.

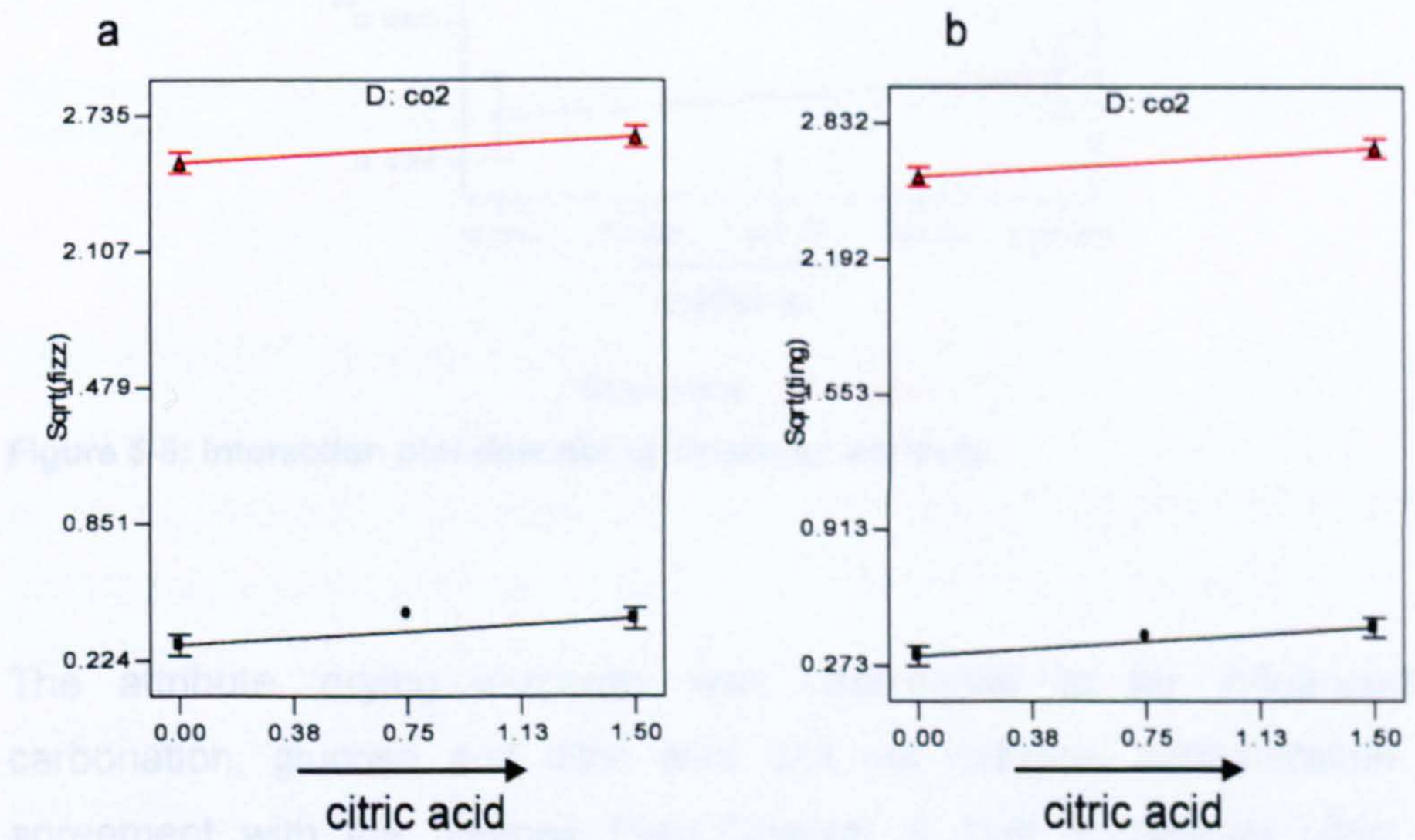
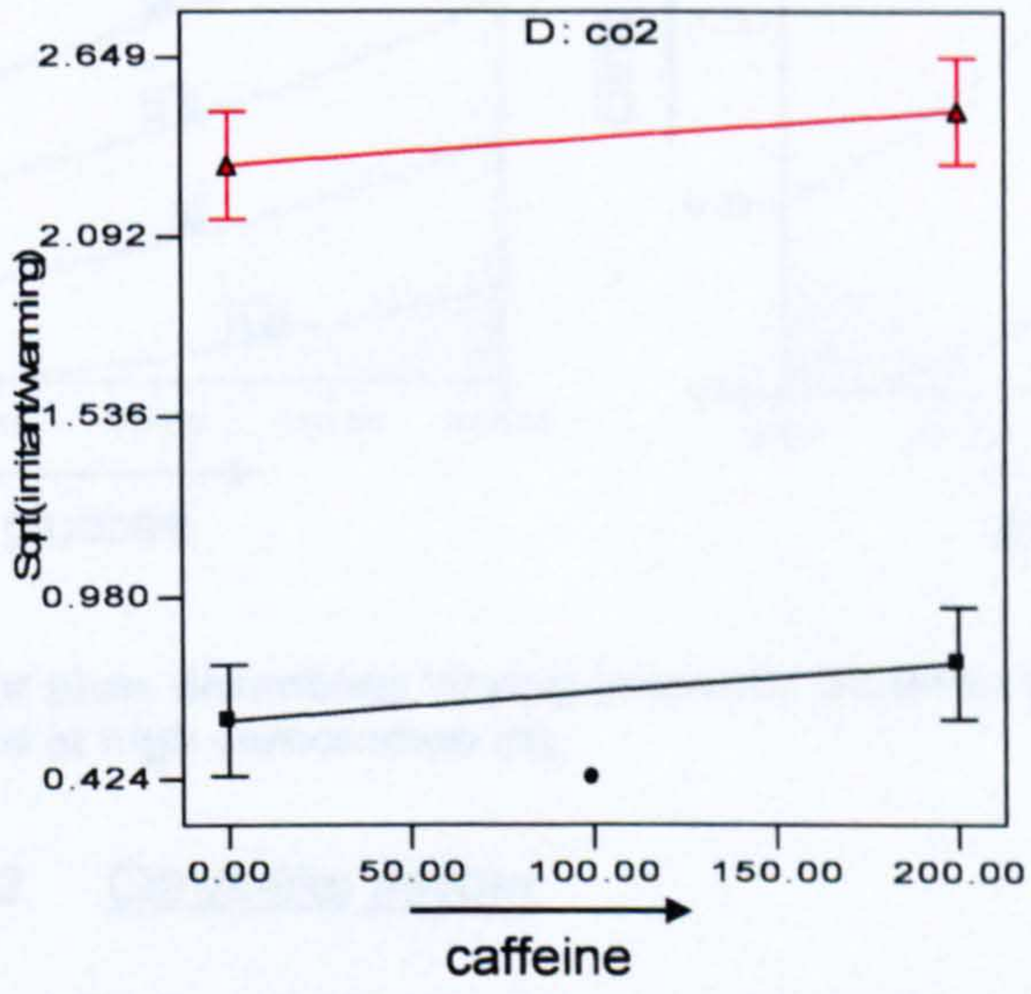


Figure 5-4: Interaction plots describing 'overall fizziness' (a) and 'tingling' (b) attributes

Caffeine was included as a significant factor in the predictive model for the attribute 'irritating' (Table 5.3:3). Again, in agreement with previous findings, carbonation level was a major determining factor for perception of this

attribute (Table 5.3:4:). Visual inspection of the interaction plot generated (Figure 5-5) indicated addition of high carbonation level increased the perception of 'irritating' significantly, in addition, an increase in caffeine concentration also increased the perception of 'irritating' but to less extent.



CO_2= none, CO_2= high

Figure 5-5: Interaction plot describing 'irritating' attribute

The attribute 'drying in-mouth' was determined to be influenced by carbonation, glucose and citric acid, but not caffeine, concentration. In agreement with the findings from Chapter 4, both increasing citric acid concentration and addition of carbonation increased the perception of 'drying', whilst increasing glucose concentration suppressed this (Figure 5-6). The decrease in weighting of the citric acid component on addition of CO_2 indicates that this factor is more influential in the absence of carbonation (Table 5.3:4:) in agreement with previous findings (Chapter 4 section 4.3.5.4). This relationship can be seen by examination of the contour plots: in the absence of CO_2 (Figure 5-6a), the magnitude of change in 'drying' on

increasing citric acid from 0-1.5g/L is ~3.5, but only ~1.5 in the presence of CO_2 (Figure 5-6b).

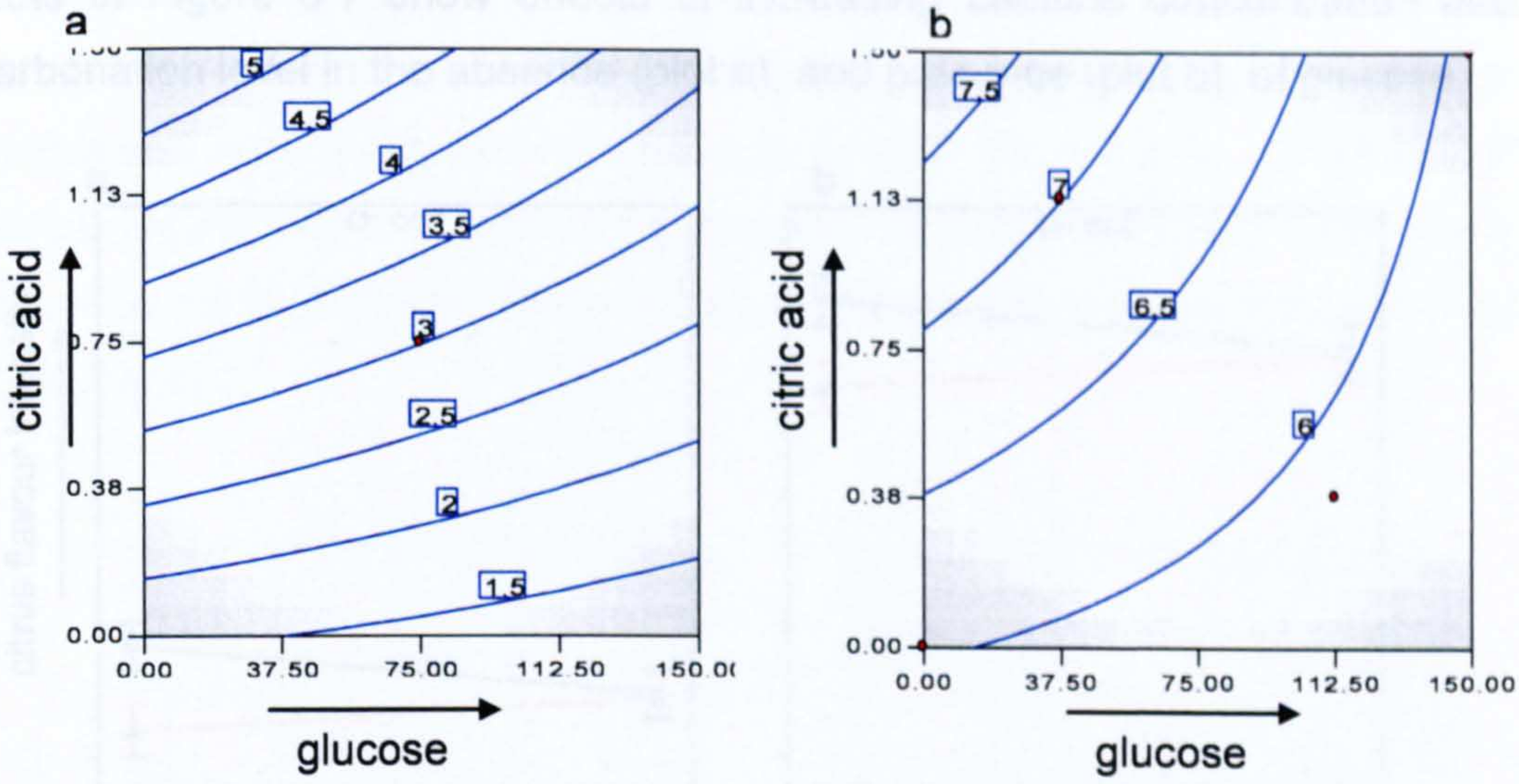


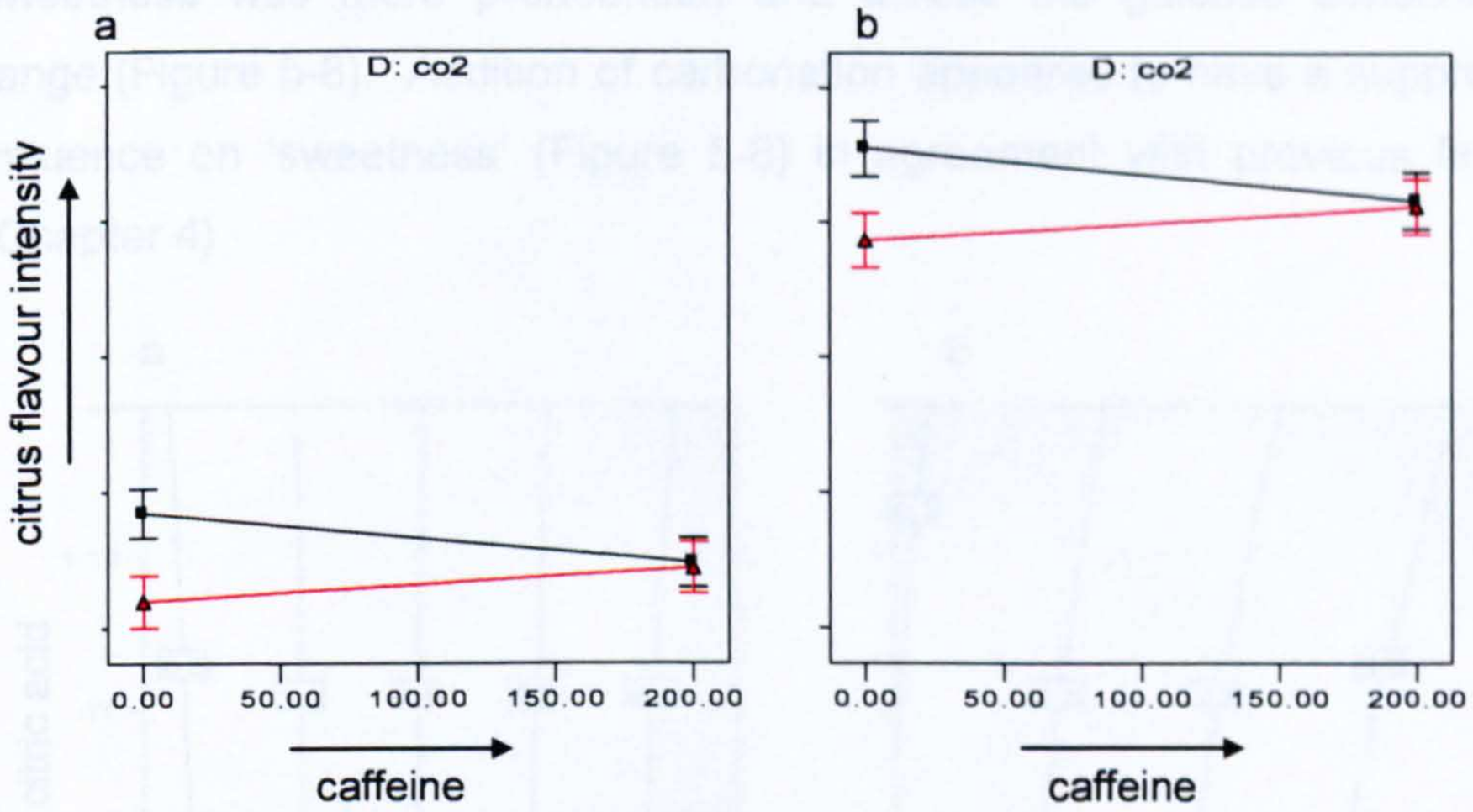
Figure 5-6: Contour plots describing 'drying in-mouth' attribute in the absence of carbonation (a) and at high carbonation (b).

5.3.4.2. Citrus-like flavour

The attribute 'citrus-like flavour' was influenced by all 4 design factors: glucose, citric acid, carbonation and caffeine. In common with findings in the previous chapter (Chapter 4) the predictive equation contained a glucose*acid interaction term and a quadratic term for glucose (*Table 5.3:4*). Assessment of contour plots revealed that the quadratic glucose term reflected a decrease in the enhancement of citrus flavour due to glucose at the mid-high glucose concentration range. This corroborated the pattern of influence of glucose on citrus flavour observed in Chapter 4.

Addition of carbonation to beverages appeared to slightly suppress the perception of citrus flavour as indicated by a reduced weighting for the intercept term in the predictive equation (Table 5.3:4). Interestingly, the influence of caffeine on this attribute appeared to be dependant on carbonation level. In the absence of carbonation, the caffeine factor has a positive weighting in the predictive equation, indicating that increasing

caffeine concentration increased perception of citrus flavour, an effect which is reversed in the presence of the high level of CO_2 (Table 5.3:4). Interaction plots in Figure 5-7 show effects of increasing caffeine concentration and carbonation level in the absence (plot a), and presence (plot b), of glucose.



a= no glucose, 0.75g/L citric acid **b**= 150g/L glucose, 0.75g/L citric acid

CO_2= none, CO_2= high

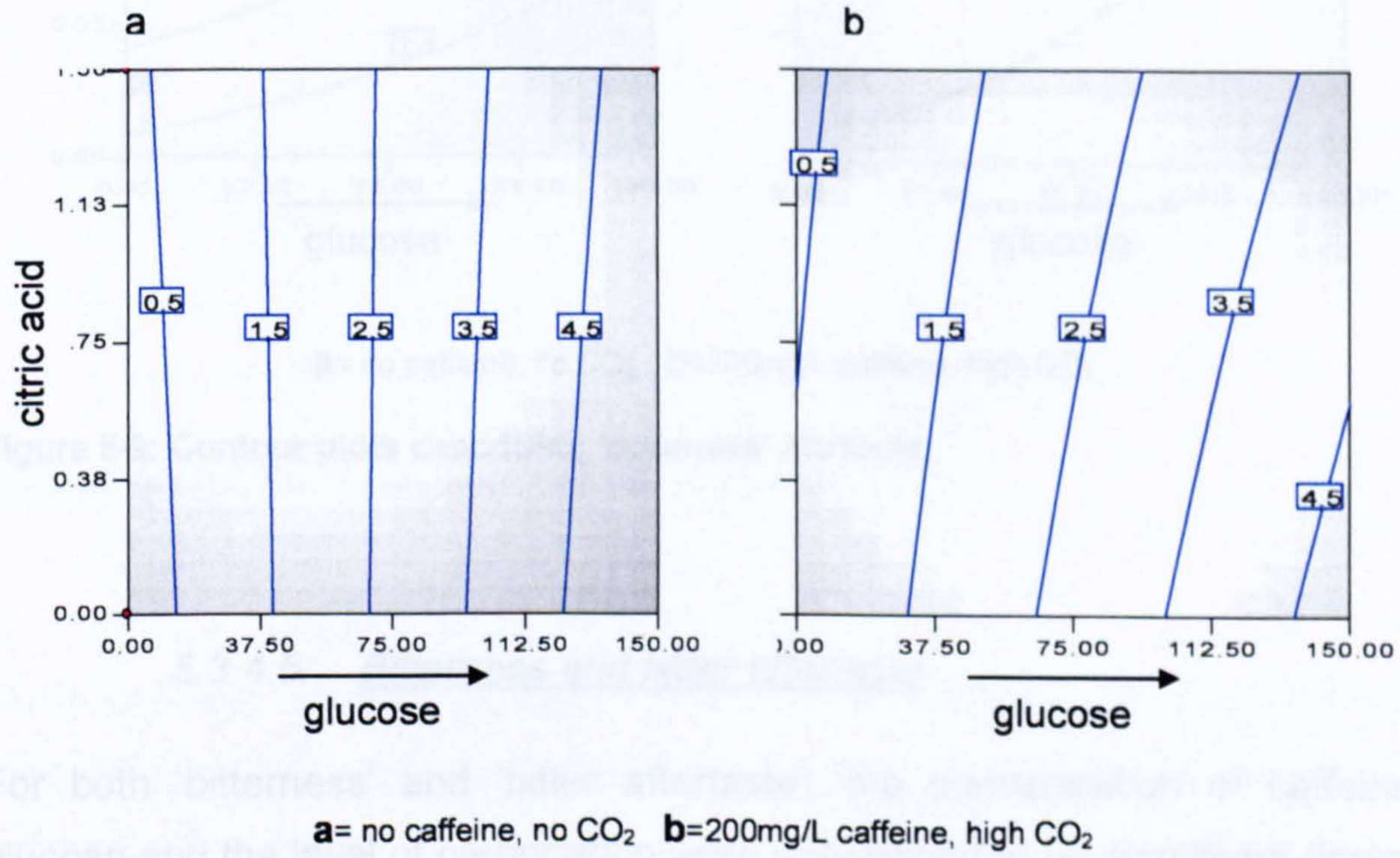
Figure 5-7: Interaction plots describing 'citrus flavour' attribute

It should be noted, however, that any modification of caffeine concentration would have only a minimal impact on 'citrus-like flavour' as the weightings for this factor are comparatively small (1.5 and 1.0 for none and high CO_2 levels respectively). Multiple comparison test results also indicated no significant differences in citrus flavour intensity between samples containing differing amounts of caffeine, in the presence of glucose (Table 5.3:1). This highlights the need for caution when interpreting predictive model equations and the necessity for examination of other statistical analysis (ANOVA and multiple comparison tests) for clarification.

5.3.4.3. Sweetness

The perceived intensity of 'sweetness' increased, as expected, with increasing glucose concentration. Citric acid had a minimal influence on

sweetness and effects of this factor were dependant on the concentration of glucose and caffeine. In the absence of caffeine, citric acid slightly suppressed sweetness of glucose levels >75g/L, whilst in the presence of high concentrations of caffeine, the suppressive effect of citric acid on sweetness was more pronounced and across the glucose concentration range (Figure 5-8). Addition of carbonation appeared to have a suppressive influence on 'sweetness' (Figure 5-8) in agreement with previous findings (Chapter 4)



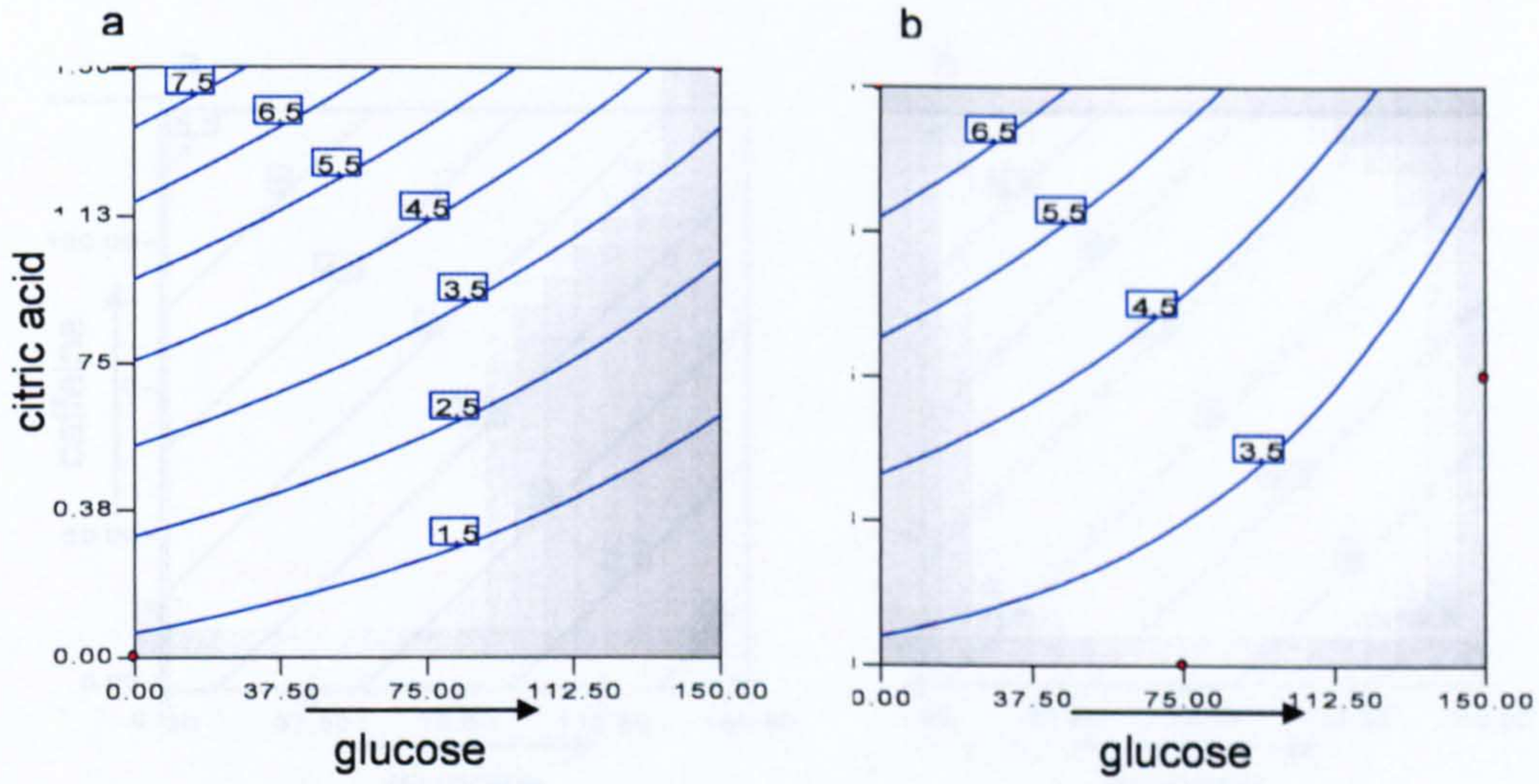
a= no caffeine, no CO_2 **b**=200mg/L caffeine, high CO_2

Figure 5-8: Contour plots describing 'sweetness' attribute

5.3.4.4. Sourness

Perception of 'sourness' was influenced by all design factors, some of which were dependant on one another as indicated by the many interaction terms included in the predictive equation (Table 5.3:4). Interpretation of this complex model showed, on the whole, 'sourness' was increased by increasing citric acid, an effect which was enhanced by carbonation and suppressed by increasing glucose concentration. The suppressive influence of glucose was greater when carbonated. Increasing caffeine concentration

appeared to have a suppressive impact on perception of 'sourness' but this effect was dependant on presence of carbonation.



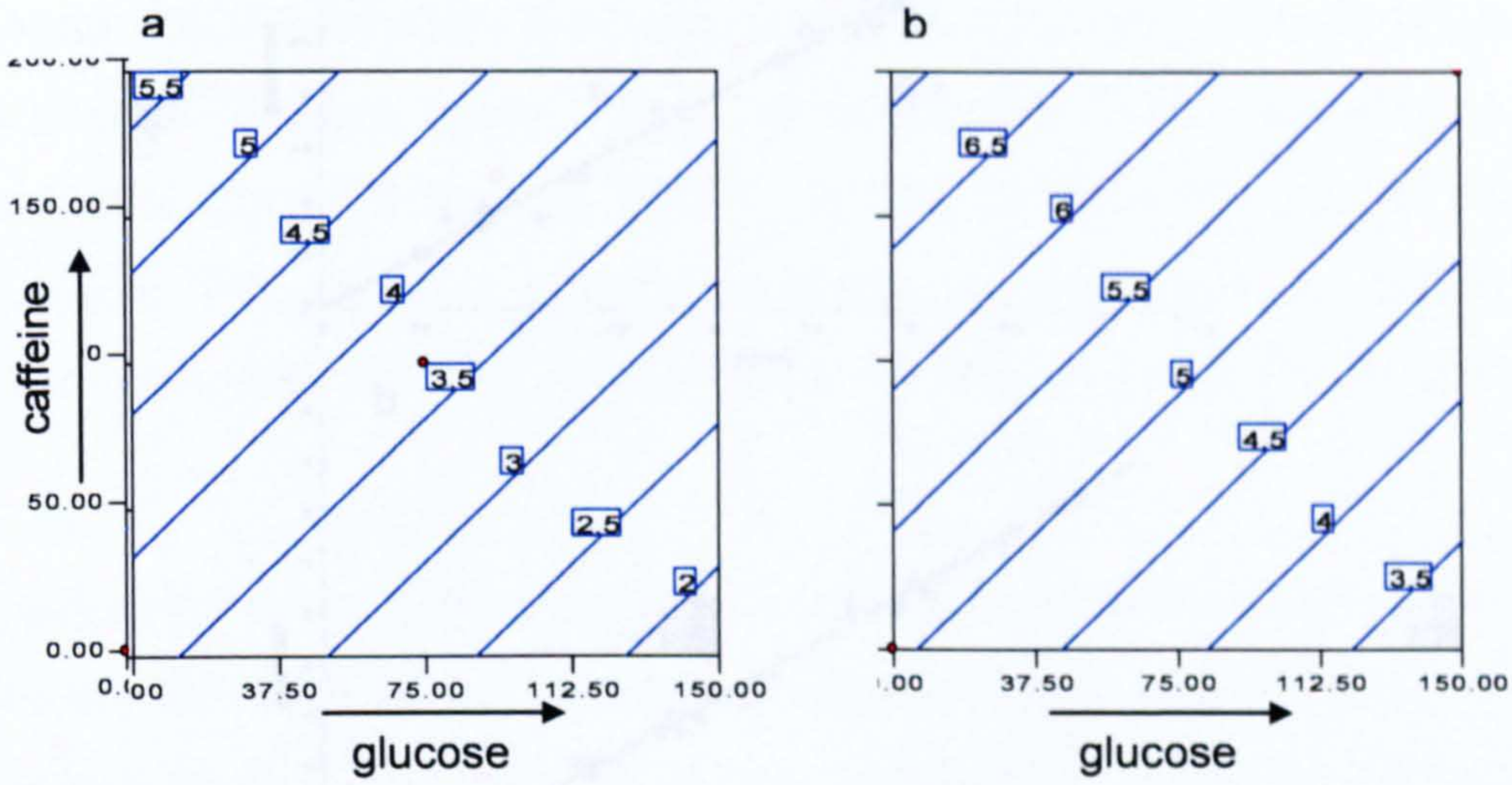
a= no caffeine, no CO_2 **b**=200mg/L caffeine, high CO_2

Figure 5-9: Contour plots describing 'sourness' attribute

5.3.4.5. Bitterness and bitter aftertaste

For both 'bitterness' and 'bitter aftertaste', the concentration of caffeine, glucose and the level of carbonation were determined to be significant design factors influencing perception of these attributes (Table 5.3:3). As expected, increasing concentration of the bitter taste compound caffeine, resulted in increases in bitterness and bitter aftertaste, an effect enhanced by the addition of carbonation. Increasing concentrations of glucose appeared to suppress the bitterness of caffeine in a linear manner across the range of caffeine and glucose concentrations examined as indicated by the contour plot (Figure 5-10). Carbonation appeared to have an enhancing influence on bitterness and bitter aftertaste. This is in agreement with the previous observation in a non-caffeinated system that addition of carbonation resulted in an increase in bitter aftertaste (Chapter 4). This supports findings by

Cometto-Muniz (Comettomuniz *et al.* 1987) suggesting CO_2 enhances bitterness of quinine sulphate at low concentrations of the bitter tastant.



a=without carbonation **b**= with carbonation

Figure 5-10: Contour plots describing 'bitterness' attribute.

5.3.5. Model validation

As in previous chapters (Chapter 3 and 4), the reliability of the predictive models generated was assessed by correlation between actual scores and model predicted scores.

Experimental scores were plotted against scores calculated by the predictive models for each attribute. In addition, intensity scores from the sensory evaluation of a separate set of samples, taken from within the design space but not included for generation of the predictive models, were plotted against their model predicted scores. Excellent correlation between actual and predicted intensity ratings was observed for each of the attributes examined, both within the experimental and validation datasets. Examples of graphs are shown in for 'citrus-like flavour' (a), 'sourness' (b) and 'bitterness' (c).

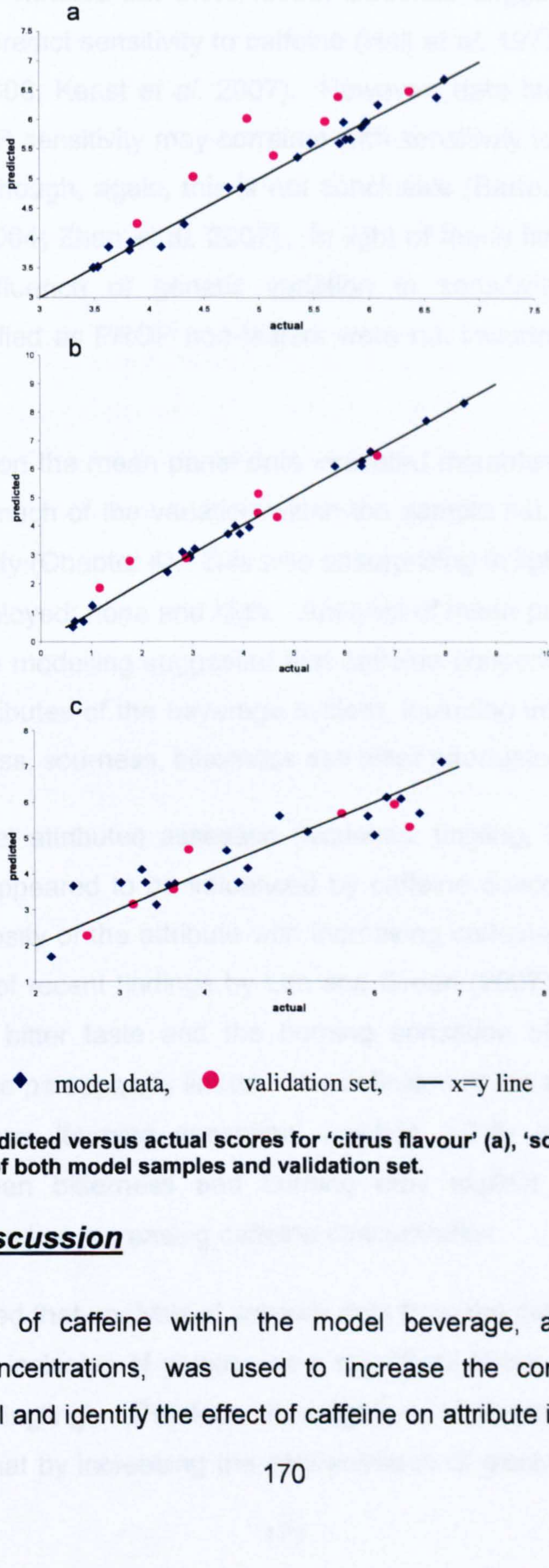


Figure 5-11: Predicted versus actual scores for 'citrus flavour' (a), 'sourness' (b) and 'bitterness' (c) of both model samples and validation set.

5.4. Discussion

The inclusion of caffeine within the model beverage, at commercially applicable concentrations, was used to increase the complexity of the sensory model and identify the effect of caffeine on attribute intensity scores.

Studies examining the relationship between sensitivity to caffeine and PROP taster status are variable but more recent evidence suggests sensitivity to PROP may not predict sensitivity to caffeine (Hall *et al.* 1975; Ly *et al.* 2001; Hansen *et al.* 2006; Keast *et al.* 2007). However, data has been reported suggesting PROP sensitivity may correlate with sensitivity to other taste and irritant stimuli although, again, this is not conclusive (Bartoshuk *et al.* 1998; Prescott *et al.* 2004; Zhao *et al.* 2007). In light of these findings, to reduce the possible influence of genetic variation in sensitivity to bitterness, assessors classified as PROP non-tasters were not included in the profiling panel.

PCA performed on the mean panel data indicated mouthfeel attributes were responsible for much of the variation within the sample set, in common with the previous study (Chapter 4). This was unsurprising in light of the levels of carbonation employed; none and high. Analysis of mean panel data and the use of predictive modelling suggested that caffeine concentration influenced a number of attributes of the beverage system, including irritating, citrus-like flavour, sweetness, sourness, bitterness and bitter aftertaste.

Of the mouthfeel attributes assessed (fizziness, tingling, irritating, drying), only 'irritating' appeared to be influenced by caffeine concentration, with an increase in intensity of the attribute with increasing caffeine level. This is of interest in light of recent findings by Lim and Green (2007). These authors suggested that bitter taste and the burning sensation of the oral irritant capsaicin may be perceptually linked. The definition of the attribute 'irritating' included the term 'burning sensation' (section 5.2.4) and a perceptual similarity between bitterness and burning may explain the increase in 'irritating' observed on increasing caffeine concentration.

It should be noted that analysis of sensory data from the current investigation did not result in inclusion of glucose as a significant factor in influencing the perception of 'tingling'. Previous investigation of the carbonated model system found that by increasing the concentration of glucose the perception

of 'tingling' was reduced (Chapter 4). The inconsistency between investigations may in part be due to the inclusion of only the highest carbonation level in the current model, whereas previously two levels of carbonation were examined. Review of the previous data indicated the influence of glucose on the attribute 'tingling', was greater at the lower level of carbonation and it is possible than this effect may not reach significance in the current model where only the higher CO_2 level incorporated into the design.

The influence of caffeine on the flavour and taste associated attributes was, as expected, most noticeable for bitterness and bitter aftertaste (Table 5.3:4:). In agreement with Pangborn (1960) and Kamen *et al* (1961), increasing glucose concentration resulted in a suppression of perceived bitterness and data indicated, overall, a small suppressive effect of caffeine on sweetness intensity.

Increasing caffeine concentrations reduced the sourness due to citric acid, supporting the findings of Pangborn *et al* (1960). This suppressive influence did not appear to be reciprocal, citric acid was not included as a significant design factor in predictive modelling of 'bitterness' or bitter aftertaste.

Interestingly, the predictive modelling determined the influence of caffeine on citrus-like flavour was dependant on carbonation. However, data suggested that in the presence of glucose, any influence of caffeine on citrus flavour did not result in significantly perceivable differences either with or without the presence of carbonation (Table 5.3:1). This finding adds to data from Griffiths and Vernotica (2000) and Keast *et al* (2006)who found subjects were unable to significantly identify differences between caffeinated and non-caffeinated colas at caffeine concentrations commonly encountered in soft drinks.

5.5. Conclusions and summary

There is a growing market in carbonated beverages containing elevated levels of caffeine. These 'energy' drinks have been shown to have beneficial effects on performance tasks and may be useful in alleviating fatigue (Alford *et al.* 2001; Horne *et al.* 2001; Kennedy *et al.* 2004). However, extensive investigation of the influence of caffeine at these concentrations on the sensory profile of beverages has not previously been undertaken.

The current study has provided novel findings on the role of caffeine at concentrations applicable to both standard and caffeinated 'energy' style carbonated soft drinks.

Caffeine is often listed as a 'flavour enhancer' or 'flavouring' in commercial soft drinks. This study provided little support for a role of caffeine in enhancement of flavour despite inclusion of caffeine as a significant factor in predictive modelling of citrus flavour. Interpretation of the modelling data alongside multiple comparison analysis concluded that increasing caffeine concentration did not result in significant differences in citrus flavour intensity within the selected samples rated. Nevertheless, a key impact factor of caffeine is its bitter taste; consequently increasing caffeine levels would be accompanied by an increasing intensity of bitterness and bitter aftertaste attributes as observed in this study. This increase in bitterness may be responsible for the finding by Griffiths and Verontica (2000) that addition of caffeine at 0.2g/L to cola drinks resulted in identifiable differences between caffeinated and non-caffeinated samples.

Sugars have previously been shown to be effective suppressors of bitterness (Pangborn 1960; Kamen *et al.* 1961; Calvino *et al.* 1990) and findings from this study confirm glucose's ability to suppress the bitterness of caffeine. The concentration of caffeine in commercially available cola flavoured carbonated beverages has been suggested to be below the detection threshold of most consumers (Griffiths *et al.* 2000; Keast 2006) possibly due in part to the

bitter-masking effect of sugars and sweeteners. It is noteworthy that findings of Schiffman (1986) suggest the use of artificial sweeteners in diet drinks may result in potentiation of bitter taste notes, which may require additional bitter masking agents to be used.

'Energy' drinks containing elevated caffeine levels additionally contain high sugar concentrations (commonly glucose) and reports suggest this combination is important for the performance enhancing effects of these beverages (Kennedy *et al.* 2004; Scholey *et al.* 2004). In these circumstances, the high concentration of glucose may be sufficient to mask increases in bitter attributes arising from elevation of caffeine levels and data from this study supports this theory. Nonetheless, caffeine concentration was also determined to influence other attributes in the model beverage system, for example 'irritating' intensity. Investigation of hedonic responses to varying caffeine concentrations in this beverage system would enable appraisal of the influence on hedonic ratings of the perceptual effects of caffeine observed in this study.

6. General conclusions

The aim of this project was to investigate the influence of varying concentrations of common beverage ingredients (design factors) on perceptual attributes. A model beverage system was created using sugar (glucose or fructose), acid (citric acid or lactic acid), aroma volatiles (simple citrus style blend), caffeine and carbonation, at commercially relevant levels.

Beginning with a simple system (taste and aroma), and gradually increasing the complexity (inclusion of carbonation and caffeine), the effects of variation of these gustatory, olfactory and trigeminal stimuli were investigated. Both instrumental measurements, aimed at identifying physicochemical interactions between beverage components, and sensory evaluation were employed to elucidate multimodal interactions influencing perceptual attributes.

6.1. Main findings and implications

6.1.1. Taste and aroma interactions

In the non-carbonated, non-caffeinated model beverages, sensory evaluation provided clear evidence of taste and aroma interactions. Increasing the concentration of lactic acid, citric acid, (equi-sour concentrations) or fructose resulted in an enhancement of citrus flavour intensity, despite the concentration of aroma volatiles remaining constant (Chapter 3). This effect could not be accounted for by physicochemical interactions occurring within the beverage matrix, as instrumental analysis suggested little modification in aroma volatile release on varying beverage composition (Chapter 2). Previous studies (Pfeiffer *et al.* 2006) have suggested that the influence of acids on flavour perception may be dependant on the congruency between acid and aroma. Lactic acid is commonly found in dairy products whereas citric acid is characteristic of citrus fruits, and therefore a more congruent

pairing with the aroma volatiles. As a consequence, one could hypothesise that citric acid would be more influential in this system, however, data provided no evidence to support this. In Pfeiffer's study, acids were compared at equi-ratio levels but this method is unlikely to result in equi-sourness and may account for the differences observed.

6.1.2. Enhancement of flavour; a tale of two sugars

Interestingly, increasing glucose concentration resulted in a differential enhancement of citrus flavour compared to fructose. Although low concentrations of glucose enhanced citrus flavour intensity, further addition did not result in further flavour enhancement (Chapter 3). Glucose and fructose ranges were chosen to be perceptually equi-sweet so this finding would not result from perceptual differences in sweetness intensity elicited by the two sugars. Possibly the most obvious explanation of this would be related to the differing solute content required to maintain equi-sweetness. Indeed, instrumental analysis indicated a small increase in viscosity at the highest glucose concentration which may influence flavour perception via modification of mouthfeel. Further work investigating the effect of the viscosity difference on perceptual mouthfeel, and the influence of this on flavour perception is warranted. If the very small modifications in viscosity of beverages, identified in this study, are able to manipulate flavour perception, an awareness of such may be imperative in controlling the flavour profile of artificially sweetened drinks.

Alternatively, as discussed in Chapter 3, this finding may be linked to reported differences between the binding affinities of the two monosaccharides with subunits of the sweet receptor, which may consequently result in differential central processing and integration. High concentrations of natural sugars have been shown to activate the T1R3:T1R3 homodimeric sweet receptor (Zhao *et al.* 2003) and mapping studies suggest this is differentially distributed in the oral cavity to the T1R2:T1R3 heterodimeric receptor (Nelson *et al.* 2001). (Nelson *et al.*

2001). Previous work has shown distinct contributions of the two subunits T1R2 and T1R3 to the detection of sweet taste and has found glucose exhibits a higher binding affinity to the T1R2 subunit, sucrose to the T1R3 subunit (Nie *et al.* 2005). Damak *et al* (2003) showed responses to both sucrose and fructose were significantly reduced in mice without the T1R3 subunit whilst responses to glucose where unaffected. Taken together these findings suggest that fructose, like sucrose, exhibits higher binding to the T1R3 subunit. Concentration ranges were chosen such that the perception of sweetness elicited by glucose and fructose was equal, but it is possible that this perception results not only from activation of the T1R2:T1R3 receptor but also, at the higher concentrations, a T1R3:T1R3 receptor. Due to the reported differential distribution of these subunits, it is likely that innervation occurs via distinct nerve pathways (Spector *et al.* 1997; Nelson *et al.* 2001). This may have implications for processing of gustatory information particularly relevant in brain regions responsive to both taste and aroma stimuli. If the neural pathways stimulated by activation of the sweet receptor differ depending on the subunit composition of the receptor itself (due to differential oral distribution), this may result in perceptual differences as a consequence of activation of uni- or bi-modal neurons.

This novel finding regarding the influence of glucose and fructose on flavour perception, in this system, raises implications for alteration of sugar type in product formulation, even in cases when sweetness perception is unaffected. Brain imaging studies could be used to elucidate the neuronal activation in response to glucose and fructose at perceptually equi-sweet levels. This may provide useful information to clarify the hypothesised differences in receptor interaction and subsequent processing of the gustatory stimuli.

It should be noted, however, that methodological issues meant only one attribute was rated per session, a chief criticism of this first study. This type of response constraint may lead to 'dumping' bias; where lack of an appropriate response option results in assessors 'dumping' sensations into

inappropriate attribute ratings (Lawless *et al.* 1992). Consequently, the flavour enhancement effects noted on addition and increasing tastant concentration may be a result of restriction of assessor's response options within a session. It may be expected, however, that 'dumping' effects would be seen across the tastants used. In this case, the finding that glucose and fructose, despite being perceptually equi-sweet, differentially influenced flavour perception, is curious. Whilst a response constraint may explain the pattern of flavour enhancement with increasing fructose concentration, it does not fully explain the response observed on increasing glucose content.

In subsequent studies of carbonated and caffeinated beverages, the technique of sensory profiling was considered more appropriate for evaluation of the more complex systems, and concurrently reduced any influence of dumping effects. It is encouraging to note that under these conditions, the effects of glucose and fructose on rated intensity of citrus flavour supported the findings of the previous study.

6.1.3. Influence of carbonation

Evaluation of the carbonated model beverages included rating of a number of mouthfeel attributes which, although modified by alteration in concentration of tastants, were mainly attributable to the presence and level of CO_2, (Chapter 4). Once more, differences in the influence of the two sugars could be identified. Increasing glucose concentration suppressed the intensity of 'tingling' and 'irritant' sensations, whilst increasing fructose concentration did not modify perception of these attributes. This suggested the presence of trigeminal-taste interactions, which appeared to be sugar specific. Whether or not suppression of the 'tingling' of a carbonated beverage is a desirable attribute remains to be seen. Further investigation, including hedonic rating and preference testing, would be useful in establishing if beverage formulation with 'less bite' would be advantageous. In these systems, varying the concentration of either glucose or fructose provided no evidence to suggest an effect on 'overall fizziness' perception. However, in light of the

use of artificial sweeteners as a low calorie replacement for bulk sugars, the impact of solute concentration on number and size of bubbles in carbonated beverages should be considered for further research.

This project has expanded previous studies and provided a comprehensive assessment of the influence of carbonation on sensory attributes of a model beverage. In agreement with previous research (Comettomuniz *et al.* 1987; Cowart 1998), data that confirmed addition of CO_2 enhanced the perception of sourness, and that this enhancement followed a CO_2 level dependant pattern. In both caffeinated and non-caffeinated beverages, the addition of carbonation decreased the sweetness of both fructose and glucose, confirming previously inconclusive evidence of the sweetness suppressing effect of CO_2 (Comettomuniz *et al.* 1987; Odake 2001).

The addition of carbonation to samples resulted in a decrease in headspace volatile content, compared to non-carbonated counterparts. As previously discussed (Chapter 2) this may be due to interactions between the dissolved CO_2 and the volatile molecules, but, perhaps more likely, is a consequence of the sampling technique used. Published literature suggests that the trigeminal system is able to exert an inhibitory influence on the olfactory system (Cain *et al.* 1980; Brand 2006). In this study, however, carbonation caused little modification of citrus flavour ratings in this system (Chapter 4). The concentration of aroma volatiles in the model beverages was relatively low and the effect of carbonation on higher concentrations of volatiles deserves further investigation. Opening a carbonated beverage results in release of CO_2 from solution and creates a highly dynamic system with initial effervescence becoming muted over time. Time intensity methodology, in combination with a liquid pump system, such as the Dynataste system used by Hort and Hollowood (2004), would provide a temporal profile of the influence of carbonation, with the ability to simultaneously vary other factors, such as volatile content. Examination of perception in such a system would

supply valuable information pertaining to the influence of carbonation on attributes such as sourness and sweetness over time.

6.1.4. Influence of caffeine

Inclusion of caffeine as a design factor in the model beverage system enabled investigation of its influence at levels consistent with standard and 'energy' drink formulations (Chapter 5). Findings suggested that caffeine concentration did not significantly influence perception of mouthfeel attributes but was, unsurprisingly, a factor determining bitterness and bitter aftertaste (Chapter 5). Furthermore, at the concentrations examined, no evidence was provided to suggest that caffeine influenced perception of citrus flavour. Previous research by Keast and Riddell (2006), using a panel of trained assessors, found that caffeine detection thresholds in sweetened solutions were below the level of caffeine used in commercial soft drinks. Despite this, the same authors reported that assessors were unable to discriminate between caffeinated and non-caffeinated colas. They suggested that the complexity of the carbonated drink masked any contribution of caffeine to flavour. Although, the current project did not specifically address whether beverage samples containing caffeine could be identified from those without, the lack of influence of caffeine concentration on citrus flavour ratings imply that differences would not be attributable to a flavour enhancing effect. This finding is in agreement with Keast and Riddell (2006) and also similar observations reported by Griffiths and Vernotica (2000), but expands these to include a non-cola flavoured system.

6.2. Use of predictive models

The predictive models generated from the sensory evaluation data were fundamental in interpreting the effect of varying the concentration of design factors on perceptual attributes. Validation of these mathematical models using novel samples provided confidence that they were robust and could be reliably used for prediction of new data. Interaction and contour plots allowed

easy visualisation of the complex models, more specifically, modification of attributes across the design space. Whilst this type of data analysis and visualisation is invaluable for interpretation, care should be taken, to prevent 'over-interpretation' of such output; interrogation of raw data and other statistical measures, such as ANOVA and multiple comparison tests, should also be used.

6.3. A wider context

Finally, it is worth mentioning that recent reports suggest that the market for high sugar, carbonated drinks is declining in favour of 'healthier' alternatives, such as juices and water (International 2007). It should be noted that carbonation is not 'unhealthy' per se, rather that the large amounts of sugars and acids added to carbonated beverages make these drinks calorific and tooth damaging. Dietary issues relating to carbonated beverage consumption are, in part, due to the more nutritional drinks they replace (milk, fruit juices), in addition to the calories they provide with little associated nutritional benefit. Nonetheless, incorporation of CO_2 adds tingle, sparkle, and fizzy sensations, which give beverages and foods novelty and may increase appeal. This novelty, combined with use of artificial sweeteners and addition of vitamins and minerals, could produce appealing beverages which have a unique market position. The distinctive character of carbonation has already been utilised to make nutritionally important products more attractive to children, for example fizzy fruit (Fizzy Fruit ^{TM} Company, USA) and carbonated milk (e-Moo, Mac Farms Inc, USA).

In a similar manner, the inclusion of caffeine in beverages can be useful in certain circumstances. Low to moderate doses (50-300mg) of caffeine have been shown to increase alertness, ability to concentrate and reduce fatigue (Nehlig 1999). These effects are especially beneficial in low alertness situations, such as night work or distance driving (Brice *et al.* 2001), thus raising implications for dealing with safety concerns. As detailed in Chapter

5, a number of 'energy' drinks, containing up to 80mg per serving of caffeine, have come onto the market in recent years and evidence suggests that these improve performance in a number of tasks, and could be beneficial in these low alertness paradigms (Horne *et al.* 2001). However, the common inclusion of lower concentrations of caffeine, solely as a 'flavouring', is somewhat more questionable, as previously discussed.

6.4. Summary

Findings of these investigations, using a model beverage system, supply information on multimodal interactions impacting on the sensory attributes of a carbonated beverage. These can be used to identify the influence of varying the concentrations of beverage constituents on perceptual attributes. It is clear that the addition or modification of sugar, acid and CO_2 in a beverage will influence more than simply sweetness, sourness and bubbles. For example, decreasing glucose concentration to lower calorific content, will impact not only on perception of sweetness, but also perception of flavour, sourness, bitterness and mouthfeel attributes (Chapter 4). To investigate the relationship between liking and sensory perception in this model system, a further study, correlating consumer liking with the sensory attribute ratings generated in this project has been undertaken (Silva 2007).

Whilst the findings of this project relate specifically to the citrus flavoured model beverage system, they offer novel evidence of perceptual relationships between common beverage ingredients. Many commercial beverages contain additional ingredients such as colourings, which have previously been shown to influence sensory perception (Petit *et al.* 2007). Nevertheless, there are a number of less complex beverages, such as carbonated, flavoured waters, for which the approach and findings of this project would have greater direct relevance.

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Appendix 1: ANOVA results from analysis of predictive models for attributes in Model 1-4

Attribute	MODEL 1					
		Sum of Squares	DF	Mean Square	F Value	Prob > F
citrus flavour	Model	36155 80	5	7231 16	95 13	< 0 0001
	G	3028 87	1	3028 87	39 84	< 0 0001
	LA	24423 18	1	24423.18	321 28	< 0.0001
	AV	4033 18	1	4033 18	53 06	< 0 0001
	G^2	710 35	1	710 35	9 34	0 0054
	G*LA	365 70	1	365 70	4 81	0.0382
	Residual	1824 41	24	76 02		
	Lack of Fit	655 28	8	81.91	1.12	0 4004
	Pure Error	1169 13	16	73 07		
	Cor Total	37980.22	29			

MODEL 2					
	Sum of Squares	DF	Mean Square	F Value	Prob > F
Model	35806 70	4	8951 68	110 11	< 0 0001
F	4121 01	1	4121 01	50 69	< 0 0001
LA	23762 86	1	23762 86	292 29	< 0 0001
AV	2905.99	1	2905 99	35.74	< 0 0001
F*LA	947.16	1	947.16	11 65	0 0022
Residual	2032 46	25	81 30		
Lack of Fit	1104 49	9	122 72	2.12	0 0915
Pure Error	927 96	16	58 00		
Cor Total	37839 16	29			

Attribute	MODEL 3					
		Sum of Squares	DF	Mean Square	F Value	Prob > F
citrus flavour	Model	34431 59	5	6886 32	140 36	< 0 0001
	G	3068 28	1	3068 28	62 54	< 0 0001
	CA	19130 75	1	19130 75	389 93	< 0 0001
	AV	3955.22	1	3955 22	80 62	< 0 0001
	G^2	843 86	1	843 86	17 20	0 0003
	G*CA	652 07	1	652 07	13 29	0 0010
	Residual	1471 87	30	49 06		
	Lack of Fit	183 52	8	22 94	0.39	0 9133
	Pure Error	1288 35	22	58 56		
	Cor Total	35903 47	35			
sourness		Sum of Squares	DF	Mean Square	F Value	Prob > F
	Model	90946 77	5	18189 35	698 50	< 0 0001
	G	2241.18	1	2241.18	85 82	< 0 0001
	CA	52097.32	1	52097.32	1994 88	< 0 0001
	AV	401 52	1	401.52	15 37	0.0008
	CA^2	445 81	1	445 81	17.07	0 0005
	G*CA	5006.15	1	5006 15	191.69	< 0 0001
	Residual	522 31	20	26 12		
	Lack of Fit	213 42	8	26 68	1.04	0 4611
	Pure Error	308 89	12	25 74		
	Cor Total	91469 08	25			
sweetness		Sum of Squares	DF	Mean Square	F Value	Prob > F
	Model	109218.70	5	21843.74	232 85	< 0 0001
	G	107893.85	1	107893.85	1150 12	< 0 0001
	CA	792 53	1	792 53	8 45	0 0103
	G^2	539 60	1	539 60	5.75	0 0290
	CA^2	720 77	1	720 77	7 68	0 0136
	G*CA	1609 83	1	1609 83	17.16	0 0008
	Residual	1500 98	16	93 81		
	Lack of Fit	740.75	8	92.59	0 97	0 5142
	Pure Error	760 22	8	95 03		
	Cor Total	110719 68	21			

MODEL 4					
	Sum of Squares	DF	Mean Square	F Value	Prob > F
Model	43872 61	3	14624 20	98 92	< 0 0001
A	7647.97	1	7647 97	51.73	< 0 0001
B	31869 62	1	31869 62	215 58	< 0 0001
C	5955 49	1	5955 49	40 29	< 0 0001
Residual	3843 66	26	147 83		
Lack of Fit	2782.14	9	309 13	4 95	0 0023
Pure Error	1061 52	17	62 44		
Cor Total	47716 27	29			
	Sum of Squares	DF	Mean Square	F Value	Prob > F
Model	91634 41	4	22908 60	220 01	< 0 0001
F	1656 24	1	1656 24	15 91	0 0010
CA	56003 87	1	56003 87	537 85	< 0 0001
CA^2	479 82	1	479 82	4 61	0 0465
F*CA	4064 69	1	4064 69	39 04	< 0 0001
Residual	1770 12	17	104 12		
Lack of Fit	700 05	8	87 51	0 74	0 6618
Pure Error	1070 07	9	118 90		
Cor Total	93404 53	21			
	Sum of Squares	DF	Mean Square	F Value	Prob > F
Model	109136 21	4	27284 05	673 26	< 0 0001
F	104920 51	1	104920 51	2589 00	< 0 0001
CA	55 02	1	55 02	1 36	0 2649
F^2	2286 36	1	2286 36	56 42	< 0 0001
F*CA	715 38	1	715 38	17 65	0 0010
Residual	526 83	13	40 53		
Lack of Fit	299 25	8	37.41	0 82	0 6175
Pure Error	227.58	5	45 52		
Cor Total	109663 04	17			

Appendix 2: ANOVA results from analysis of predictive models for attributes in Model G

MODEL G

Attribute		Sum of		Mean	F	
overall fizziness	Source	Squares	DF	Square	Value	Prob > F
	Model	15 30	2	7 65	191 23	< 0 0001
	CO_2	15 30	2	7 65	191 23	< 0 0001
	Residual	0 60	15	0 04		
	Lack of Fit	0 51	12	0 04	1 51	0 4101
	Pure Error	0 09	3	0 03		
	Cor Total	15.90	17			
tingling		Sum of		Mean	F	
	Source	Squares	DF	Square	Value	Prob > F
	Model	15 30	8	1 91	112 60	< 0 0001
	G	0 15	1	0 15	9 03	0 0148
	CA	0 07	1	0 07	4 09	0 0739
	CO_2	14 97	2	7 48	440 54	< 0 0001
	G*CO_2	0.17	2	0 09	5 06	0 0338
	CA*CO_2	0.15	2	0 08	4 54	0 0433
	Residual	0 15	9	0 02		
	Lack of Fit	0 08	6	0.01	0 58	0.7391
	Pure Error	0 07	3	0.02		
	Cor Total	15 46	17			
drying in mouth		Sum of		Mean	F	
	Source	Squares	DF	Square	Value	Prob > F
	Model	8 40	8	1 05	51 05	< 0 0001
	G	1 34	1	1 34	65 10	< 0 0001
	CA	1 45	1	1 45	70 58	< 0 0001
	CO_2	4 96	2	2 48	120 58	< 0 0001
	G*CO_2	0 21	2	0.11	5 12	0 0328
	CA*CO_2	1 03	2	0 52	25 08	0 0002
	Residual	0 19	9	0 02		
	Lack of Fit	0 17	6	0 03	6 48	0 0769
	Pure Error	0 01	3	0 00		
	Cor Total	8 59	17			
irritant		Sum of		Mean	F	
	Source	Squares	DF	Square	Value	Prob > F
	Model	59 54	9	6 62	89 36	< 0 0001
	G	0 27	1	0 27	3 66	0 0921
	CA	0 98	1	0 98	13 28	0 0066
	CO_2	54 13	2	27.07	365 59	< 0 0001
	G^2	1 37	1	1 37	18 49	0 0026
	G*CO_2	2 06	2	1 03	13 94	0 0025
	CA*CO_2	1 27	2	0 63	8 56	0 0103
	Residual	0 59	8	0 07		
	Lack of Fit	0 31	5	0 06	0 66	0 6833
	Pure Error	0 28	3	0 09		
	Cor Total	60 13	17			
citrus flavour		Sum of		Mean	F	
	Source	Squares	DF	Square	Value	Prob > F
	Model	20 45	9	2 27	25 93	< 0 0001
	G	5 23	1	5 23	59 68	< 0 0001
	CA	9 80	1	9 80	111 83	< 0 0001
	CO_2	0 75	2	0 38	4 29	0 0543
	G^2	0 74	1	0 74	8 40	0 0200
	G*CO_2	0 89	2	0 45	5 08	0 0377
	CA*CO_2	0 98	2	0 49	5 56	0 0306
	Residual	0 70	8	0 09		
	Lack of Fit	0 26	5	0 05	0 35	0 8541
	Pure Error	0 44	3	0 15		
	Cor Total	21 16	17			

Attribute		Sum of		Mean	F	
sweetness	Source	Squares	DF	Square	Value	Prob > F
	Model	178 92	7	25 56	277 97	< 0 0001
	G	171 65	1	171 65	1866 71	< 0 0001
	CA	2 61	1	2 61	28 40	0 0003
	CO_2	2 48	2	1 24	13 51	0 0014
	G*CA	1 48	1	1 48	16 06	0 0025
	G*CO_2	2 13	2	1 07	11 60	0 0025
	Residual	0 92	10	0 09		
	Lack of Fit	0 80	7	0 11	2 86	0 2091
	Pure Error	0 12	3	0 04		
	Cor Total	179 84	17			
sourness		Sum of		Mean	F	
	Source	Squares	DF	Square	Value	Prob > F
	Model	7.41	6	1.23	32 67	< 0 0001
	G	2.18	1	2 18	57 65	< 0 0001
	CA	3 45	1	3 45	91 25	< 0 0001
	CO_2	1 40	2	0 70	18 55	0 0003
	CA*CO_2	0 82	2	0 41	10 82	0 0025
	Residual	0 42	11	0 04		
	Lack of Fit	0 38	8	0 05	3 73	0 1533
	Pure Error	0 04	3	0 01		
	Cor Total	7 82	17			
bitter aftertaste		Sum of		Mean	F	
	Source	Squares	DF	Square	Value	Prob > F
	Model	28 30	3	9 43	19 98	< 0 0001
	G	15 53	1	15 53	32 91	< 0 0001
	CO_2	13 71	2	6 85	14 52	0 0004
	Residual	6 61	14	0 47		
	Lack of Fit	6 36	11	0 58	7 15	0 0659
	Pure Error	0 24	3	0 08		
	Cor Total	34 90	17			
acidic aftertaste		Sum of		Mean	F	
	Source	Squares	DF	Square	Value	Prob > F
	Model	68 85	7	9 84	55 74	< 0 0001
	G	16 50	1	16 50	93 51	< 0 0001
	CA	36 97	1	36 97	209 50	< 0 0001
	CO_2	11 91	2	5 95	33 75	< 0 0001
	G*CA	4 81	1	4 81	27 28	0 0004
	CA*CO_2	1 59	2	0 80	4 51	0 0401
	Residual	1 76	10	0 18		
	Lack of Fit	1 68	7	0 24	8 47	0 0533
	Pure Error	0 08	3	0 03		
	Cor Total	70 61	17			
drying aftertaste		Sum of		Mean	F	
	Source	Squares	DF	Square	Value	Prob > F
	Model	0 48	8	0 06	141 30	< 0 0001
	G	0 07	1	0 07	155 18	< 0 0001
	CA	0 10	1	0 10	225 94	< 0 0001
	CO_2	0 25	2	0 13	301 12	< 0 0001
	G*CO_2	0 02	2	0 01	19 81	0 0005
	CA*CO_2	0 07	2	0 04	86 81	< 0 0001
	Residual	0 00	9	0 00		
	Lack of Fit	0 00	6	0 00	0 65	0 7036
	Pure Error	0 00	3	0 00		
	Cor Total	0 48	17			

Appendix 3: ANOVA results from analysis of predictive models for attributes in Model F

MODEL F

Attribute	Source	Sum of Squares	DF	Mean Square	F Value	Prob > F
overall fizziness	Model	13 83	4	3 46	462 72	< 0 0001
	CA	0 01	1	0 01	1 82	0 2023
	CO_2	13 63	2	6 82	912 37	< 0 0001
	CA^2	0 05	1	0 05	6 34	0 0270
	Residual	0 09	12	0 01		
	Lack of Fit	0 08	9	0 01	1 79	0 3439
	Pure Error	0 01	3	0 00		
	Cor Total	13 92	16			
tingling	Model	14 78	4	3 70	374 42	< 0 0001
	CA	0 05	1	0 05	4 77	0 0496
	CO_2	14 47	2	7 24	733 12	< 0 0001
	CA^2	0 05	1	0 05	5 32	0 0398
	Residual	0 12	12	0 01		
	Lack of Fit	0 11	9	0 01	3 75	0 1523
	Pure Error	0 01	3	0 00		
	Cor Total	14 90	16			
drying in mouth	Model	5 21	6	0 87	120 01	< 0 0001
	F	0 32	1	0 32	43 97	< 0 0001
	CA	1 10	1	1 10	152 07	< 0 0001
	CO_2	2 76	2	1 38	190 60	< 0 0001
	$CA*CO_2$	0 51	2	0 25	35 22	< 0 0001
	Residual	0 07	10	0 01		
	Lack of Fit	0 04	7	0 01	0 43	0 8416
	Pure Error	0 04	3	0 01		
	Cor Total	5 28	16			
irritant	Model	67 11	4	16 78	123 55	< 0 0001
	CA	0 19	1	0 19	1 38	0 2636
	CO_2	65 70	2	32 85	241 91	< 0 0001
	CA^2	0 83	1	0 83	6 14	0 0291
	Residual	1 63	12	0 14		
	Lack of Fit	1 56	9	0 17	7 08	0 0673
	Pure Error	0 07	3	0 02		
	Cor Total	68 74	16			
citrus flavour	Model	7 46	1	7 46	19 22	0 0005
	F	7 46	1	7 46	19 22	0 0005
	Residual	5 84	15	0 39		
	Lack of Fit	5 54	12	0 46	4 69	0 1147
	Pure Error	0 30	3	0 10		
	Cor Total	13 31	16			
citrus flavour (outlier removed)	Model	10 19	2	5 09	22 40	< 0 0001
	F	9 64	1	9 64	42 41	< 0 0001
	CA	1 02	1	1 02	4 47	0 0544
	Residual	2 96	13	0 23		
	Lack of Fit	2 66	10	0 27	2 70	0 2237
	Pure Error	0 30	3	0 10		
	Cor Total	13 14	15			

Attribute	Source	Sum of Squares	DF	Mean Square	F Value	Prob > F
sweetness	Model	122 38	7	17 48	163 71	< 0 0001
	F	115 04	1	115 04	1077 28	< 0 0001
	CA	1 61	1	1 61	15 04	0 0037
	CO_2	0 63	2	0 31	2 93	0 1047
	F*CA	1 56	1	1 56	14 65	0 0040
	F*CO_2	1 84	2	0 92	8 63	0 0081
	Residual	0 96	9	0 11		
	Lack of Fit	0 63	6	0 11	0 97	0.5579
	Pure Error	0 33	3	0.11		
	Cor Total	123 34	16			
sourness	Model	54 82	9	6 09	75 60	< 0 0001
	F	10 47	1	10 47	129 96	< 0 0001
	CA	27 43	1	27 43	340 42	< 0 0001
	CO_2	8 17	2	4 09	50 73	< 0 0001
	F*CA	2 65	1	2 65	32 95	0 0007
	F*CO_2	0 77	2	0 39	4 79	0 0469
	CA*CO_2	1.35	2	0 68	8 41	0 0138
	Residual	0 56	7	0 08		
	Lack of Fit	0 17	4	0 04	0 31	0 8549
	Pure Error	0 40	3	0 13		
	Cor Total	55 39	16			
bitter aftertaste	Model	39 05	9	4 34	35 72	< 0 0001
	F	15 25	1	15 25	125 55	< 0 0001
	CA	0 09	1	0 09	0 73	0 4211
	CO_2	15 91	2	7 95	65 49	< 0 0001
	F*CA	0 47	1	0 47	3 86	0 0903
	F*CO_2	1.16	2	0 58	4 77	0 0494
	CA*CO_2	1 94	2	0 97	7 99	0 0156
	Residual	0 85	7	0 12		
	Lack of Fit	0 78	4	0 20	8 88	0 0516
	Pure Error	0 07	3	0 02		
	Cor Total	39 90	16			
acidic aftertaste	Model	53 11	4	13 28	56 48	< 0 0001
	F	4 38	1	4 38	18 62	0 0010
	CA	31 71	1	31.71	134 88	< 0 0001
	CO_2	12 95	2	6 47	27 54	< 0 0001
	Residual	2 82	12	0 24		
	Lack of Fit	2 62	9	0 29	4 30	0 1285
	Pure Error	0 20	3	0 07		
	Cor Total	55 93	16			
drying aftertaste	Model	44 80	6	7 47	87 09	< 0 0001
	F	4 38	1	4 38	51 10	< 0 0001
	CA	9 72	1	9 72	113 44	< 0 0001
	CO_2	24 12	2	12 06	140 69	< 0 0001
	CA*CO_2	2 08	2	1 04	12 15	0 0021
	Residual	0 86	10	0 09		
	Lack of Fit	0 67	7	0 10	1 55	0 3890
	Pure Error	0 19	3	0 06		
	Cor Total	45 65	16			

Appendix 4: ANOVA results from analysis of predictive models for attributes in Model Caffeine

MODEL Caffeine

Attribute	Source	Sum of Squares	DF	Mean Square	F Value	Prob > F
overall fizziness	Model	24 62	2	12 31	1919 70	< 0 0001
	CA	0 05	1	0 05	8 57	0 0094
	CO_2	24 48	1	24 48	3818 23	< 0 0001
	Residual	0 11	17	0 01		
	Lack of Fit	0 11	15	0 01	5 49	0 1648
	Pure Error	0 00	2	0 00		
	Cor Total	24 73	19			
tingling		Sum of		Mean	F	
	Source	Squares	DF	Square	Value	Prob > F
	Model	25 45	2	12 72	2304 78	< 0 0001
	CA	0 07	1	0 07	12 40	0 0026
	CO_2	25 29	1	25 29	4580 89	< 0 0001
	Residual	0 09	17	0 01		
	Lack of Fit	0 08	15	0 01	1 25	0 5333
	Pure Error	0 01	2	0 00		
	Cor Total	25 54	19			
drying in mouth		Sum of		Mean	F	
	Source	Squares	DF	Square	Value	Prob > F
	Model	87.83	5	17 57	74 59	< 0 0001
	G	5 14	1	5 14	21 83	0 0004
	CA	16 77	1	16 77	71 20	< 0 0001
	CO_2	54 90	1	54 90	233 12	< 0 0001
	G*CA	1 66	1	1 66	7 05	0 0188
	CA*CO_2	4 31	1	4 31	18 28	0 0008
	Residual	3 30	14	0 24		
	Lack of Fit	3 21	12	0 27	6 34	0 1443
	Pure Error	0 08	2	0 04		
	Cor Total	91.13	19			
irritant		Sum of		Mean	F	
	Source	Squares	DF	Square	Value	Prob > F
	Model	14 52	2	7 26	407.73	< 0 0001
	CAFF	0 10	1	0 10	5 75	0 0283
	CO_2	14 42	1	14 42	809 71	< 0 0001
	Residual	0 30	17	0 02		
	Lack of Fit	0 28	15	0 02	1 45	0 4839
	Pure Error	0 03	2	0 01		
	Cor Total	14 82	19			
citrus flavour		Sum of		Mean	F	
	Source	Squares	DF	Square	Value	Prob > F
	Model	21 15	7	3 02	106 31	< 0 0001
	G	16 16	1	16 16	568 50	< 0 0001
	CA	0 14	1	0.14	4 78	0 0492
	CAFF	0 01	1	0 01	0 25	0 6266
	CO_2	0 36	1	0 36	12 81	0 0038
	G^2	2 59	1	2 59	91 07	< 0 0001
	G*CA	1 06	1	1 06	37 18	< 0 0001
	CAFF*CO_2	0 19	1	0 19	6 84	0 0226
	Residual	0 34	12	0 03		
	Lack of Fit	0 19	10	0 02	0 26	0 9433
	Pure Error	0 15	2	0 07		
	Cor Total	21 49	19			

Attribute	Source	Sum of Squares	DF	Mean Square	F Value	Prob > F
sweetness	Model	79 18	7	11 31	736 20	< 0 0001
	G	68 52	1	68 52	4459 75	< 0 0001
	CA	0 57	1	0 57	37 14	< 0 0001
	CAFF	0 01	1	0 01	0 83	0 3797
	CO_2	0 18	1	0 18	11 72	0 0050
	G*CA	0 44	1	0 44	28 71	0 0002
	G*CO_2	1.15	1	1.15	74 82	< 0 0001
	CA*CAFF	0 25	1	0 25	16 31	0 0016
	Residual	0 18	12	0 02		
	Lack of Fit	0.17	10	0 02	3 11	0 2675
	Pure Error	0 01	2	0 01		
	Cor Total	79 37	19			
sourness		Sum of		Mean	F	
	Source	Squares	DF	Square	Value	Prob > F
	Model	119 69	10	11 97	237 36	< 0 0001
	G	19 78	1	19 78	392 29	< 0 0001
	CA	54 05	1	54 05	1071 87	< 0 0001
	CAFF	0 65	1	0 65	12 79	0 0060
	CO_2	7 48	1	7 48	148 39	< 0 0001
	CA^2	0 28	1	0 28	5 58	0 0425
	G*CA	8 15	1	8 15	161 58	< 0 0001
	G*CAFF	0 75	1	0 75	14 83	0 0039
	G*CO_2	0 43	1	0 43	8 55	0 0169
	CA*CAFF	0 30	1	0 30	5 93	0 0377
	CA*CO_2	3 34	1	3 34	66 26	< 0 0001
	Residual	0 45	9	0 05		
	Lack of Fit	0 44	7	0 06	8 66	0 1074
	Pure Error	0 01	2	0 01		
	Cor Total	120 14	19			
bitterness		Sum of		Mean	F	
	Source	Squares	DF	Square	Value	Prob > F
	Model	31 60	3	10 53	26 30	< 0 0001
	G	13 10	1	13 10	32 71	< 0 0001
	CAFF	13 58	1	13 58	33 91	< 0 0001
	CO_2	10 08	1	10 08	25 18	0 0001
	Residual	6 41	16	0 40		
	Lack of Fit	6 26	14	0 45	5 86	0 1551
	Pure Error	0 15	2	0 08		
	Cor Total	38 01	19			
bitter aftertaste		Sum of		Mean	F	
	Source	Squares	DF	Square	Value	Prob > F
	Model	33 86	3	11 29	27 94	< 0 0001
	G	11 63	1	11 63	28 80	< 0 0001
	CAFF	11 41	1	11 41	28 25	< 0 0001
	CO_2	15 08	1	15 08	37 34	< 0 0001
	Residual	6 46	16	0 40		
	Lack of Fit	6 31	14	0 45	6 02	0 1514
	Pure Error	0 15	2	0 07		
	Cor Total	40 32	19			

G glucose	F fructose	CA citric acid	AV aroma volatile level	CO_2 carbonation	CAFF caffeine